

FORM P  
(REV. 10-90)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

1599-0206P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

**09/937215**

INTERNATIONAL APPLICATION NO.

PCT/KR99/00131

INTERNATIONAL FILING DATE

March 24, 1999

PRIORITY DATE CLAIMED

NONE

## TITLE OF INVENTION

TREHALOSE SYNTHASE PROTEIN, GENE, PLASMIDS, MICROORGANISMS, AND A PROCESS FOR PRODUCING  
TREHALOSE

## APPLICANT(S) FOR DO/EO/US

LEE, Se Yong; SONG, Eun Kyung; PARK, Yearn Hung; KWON, Sang Ho; LEE, Kwang Ho;  
KIM, Chang Gyeom; LEE, Jin Ho; CHUNG, Sung Oh; JEON, Yeong Joong

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/56868
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☐ is transmitted herewith.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Photocopy)
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98, Form PTO-1449(s), and International Search Report (PCT/ISA/210) with 0 document(s).
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
  1. International Preliminary Examination Report (PCT/IPEA/409)
  2. PCT Request (PCT/RO/101)
  3. Sequence Listing (6 pages)
  4. Six (6) sheets of formal drawings
  5. Receipt concerning Deposit of Microorganisms

ATTORNEY'S DOCKET NUMBER

1599-0206P

21. ☒ The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):**

Neither international preliminary examination fee (37 CFR 1.482)  
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  
and International Search Report not prepared by the EPO or JPO. . . . . **\$1,000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....	<b>\$860.00</b>
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International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....	<b>\$710.00</b>
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International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....	\$690.00
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International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....	<b>\$100.00</b>
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**ENTER APPROPRIATE BASIC FEE AMOUNT =**

Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total Claims	9 - 20 =	0	X \$18.00

Independent Claims	3 - 3 =	0	X \$80.00
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MULTIPLE DEPENDENT CLAIM(S) (if applicable)	+ \$270.00
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<b>TOTAL OF ABOVE CALCULATIONS =</b>	<b>\$</b>	<b>1,000.00</b>
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☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.

<b>SUBTOTAL =</b>	<b>\$ 1,000.00</b>
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Processing fee of \$130.00 for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). ☐20 ☐30 +

<b>TOTAL NATIONAL FEE =</b>	<b>\$</b>	<b>1,000.00</b>
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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **\$40.00** per property +

<b>TOTAL FEES ENCLOSED =</b>	<b>\$</b>	<b>\$1,040.00</b>
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Amount to be:	\$
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refunded	\$
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a. ☒ A check in the amount of \$ 1,040.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:

**Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292**

**P.O. Box 747**

**Falls Church, VA 22040-0747**

**(703)205-8000**

**Date: September 24, 2001**

By Joseph A. Kolasch Reg No. 32,337  
Joseph A. Kolasch, #22,463

PATENT  
1599-0206P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: LEE, Se Yong et al.  
Int'l. Appl. No.: PCT/KR99/00131  
Appl. No.: NEW Group:  
Filed: September 24, 2001 Examiner:  
For: TREHALOSE SYNTHASE PROTEIN, GENE,  
PLASMIDS, MICROORGANISMS, AND A  
PROCESS FOR PRODUCING TREHALOSE

PRELIMINARY AMENDMENT

**BOX PATENT APPLICATION**

Assistant Commissioner for Patents  
Washington, DC 20231

September 24, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please insert the following paragraph before the paragraph beginning on page 1, line 1:

--This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/KR99/00131 which has an International filing date of March 24, 1999, which designated the United States of America and was published in English.- -

Please replace the paragraph beginning on page 13, line 13 with the following rewritten paragraph:

--The enzymatic reaction is conducted at pH 6.0 to 11, preferably pH 7.0 to 10, and at temperatures of 4°C to 45°C, preferably 20°C to 40°C. Maltose can be used as a substrate in a concentration of less than 50%. The trehalose synthase enzyme can be used in a pure form or in crushed cells.--

Please replace the paragraph beginning on page 16, line 19, with the following rewritten paragraph:

--The pure chromosomal DNAs isolated from *Pseudomonas stutzeri* were partially digested with restriction enzyme *Sau3AI* at 37°C for 15 to 30 minutes. The restriction enzyme was inactivated with heat and agarose gel electrophoresis was carried out to obtain 3 to 10 kb DNA fragments. As shown in Figure 5, plasmid pUC18 was digested with *BamHI* and was treated with calf intestinal phosphatase. The cleaved DNAs were mixed with 3 to 10 kb DNA fragments previously obtained and ligation with T4 DNA ligase was allowed at 15°C for 16 hours. The recombinants thus obtained were used for transofrmation. The transformation was carried out by electroporation as follows. *E coli* NM522 was cultured on LB medium for 14 to 15 hours. The resulting culture was inoculated on 1L LB so that initial absorbency became 0.07 to 0.1 at 600 nm, and then cultivation was allowed until the absorbency reached 0.8. The cells were centrifuged and suspended in 1L of HEPES [N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic

acid)] buffer solution. The cells were again centrifuged and suspended in 500 ml of cold sterile deionized distilled water. The cells were again centrifuged and suspended in 20 ml of 10% glycerol solution. The cells were again centrifuged and suspended in 2 to 3 ml of 10% glycerol solution so that the cell concentration was adjusted to  $2-4 \times 10^{10}$ /ml. The cell suspension was rapidly frozen and stored at  $-70^{\circ}\text{C}$ . The frozen cells could be used for about one month during which time their transformation frequency did not decrease.  $40\mu\text{L}$  of frozen cell suspension was thawed in ice and the restored suspension was mixed with the ligated DNA solution. The mixture was put in a gene pulser cuvette with a diameter of 0.2 cm and the capacitance and strength of electric field was fixed at 25  $\mu\text{F}$  and 12.5 kV/cm, respectively. After a single electric pulse was passed at resistance of 200 to 400  $\Omega$ , 1 ml of SOC medium was immediately added and cultured at  $37^{\circ}\text{C}$  for 1 hour. The culture was streaked on LB-ampicillin agar medium and cultivation was allowed for 24 hours to obtain at least fifty thousand colonies. These colonies were together cultured in LB broth for 2 hours. DNA was purely isolated using an alkaline lysis and the genomic library was constructed therefrom.--

Please replace the paragraph beginning on page 18, line 22, with the following rewritten paragraph:

--The plasmid pCJ104 was subjected to single, double, and triple-digest procedures using about twenty restriction enzymes,

such as *AatII*, *BamHI*, *BglII*, *SmaI*, *EcoRI*, *EcoRV*, *KpnI*, *NcoI*, *NdeI*, *PstI*, *SacI*, *SacII*, *SallI*, *SphI* and *XhoI*. DNA fragments were analyzed by electrophoresis through agarose gel and compared to construct the restriction map.--

Please replace the table beginning on page 18, line 5, with the following rewritten table:

--Table 3. Enzyme Titration

Microorganisms	Specific activity of enzyme (U*/mg of protein)	Culture Titer (U/ml of culture solution)
<i>Pseudomonas stutzeri</i> CJ38	0.1	0.023
<i>E. coli</i> ATCC35467/pUC18	0	0
<i>E. coli</i> ATCC35467/pCJ104	0.26	0.175

\*U- $\mu$ mol trehalose/minutes--

Please replace the heading beginning on page 18, line 28, with the following rewritten heading:

--Example 8--

Please replace the table beginning on page 20, line 17, with the following rewritten table:

--Table 5

Microorganisms	Specific activity of enzyme (U/mg of protein)	Culture Titer of 5 L Fermenter (U/ml of culture)
<i>E. coli</i> ATCC35467/pCJ121	0.43	-
<i>E. coli</i> ATCC35467/pCJ122	4.95	30
<i>E. coli</i> ATCC35467/pCJ123	0	-

--

In the Claims:

Please amend the claims as follows:

3. (Amended) A recombinant plasmid containing the trehalose synthase gene of claim 2.

4. (Amended) The recombinant plasmid according to claim 3 which is recombinant plasmid pCJ122.

5. (Amended) A transformed *E. coli* with the recombinant plasmid of claim 3.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

The claims have been amended to correct the dependency thereof.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By Joe M. Kolasch <sup>Reg. No. 32,334</sup>  
Jr Joseph A. Kolasch, #22,463

P.O. Box 747

Falls Church, VA 22040-0747

(703) 205-8000

JAK/kw

1599-0206P

Attachment: VERSION WITH MARKINGS TO SHOW CHANGES MADE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

A paragraph has been added before the paragraph beginning on page 1, line 1.

The paragraph beginning on page 13, line 13 has been amended as follows:

--The enzymatic reaction is conducted at pH 6.0 to [7.0] 11, preferably pH 7.0 to 10, and at temperatures of 4°C to 45°C, preferably 20°C to 40°C. Maltose can be used as a substrate in a concentration of less than 50%. The trehalose synthase enzyme can be used in a pure form or in crushed cells.--

The paragraph beginning on page 16, line 19, has been amended as follows:

--The pure chromosomal DNAs isolated from *Pseudomonas stutzeri* were partially digested with restriction enzyme *Sau3AI* at 37°C for 15 to 30 minutes. The restriction enzyme was inactivated with heat and agarose gel electrophoresis was carried out to obtain 3 to 10 kb DNA fragments. As shown in Figure 5, plasmid pUC18 was digested with *BamHI* and was treated with calf intestinal phosphatase. The cleaved DNAs were mixed with 3 to 10 kb DNA fragments previously obtained and ligation with T4 DNA ligase was allowed at 15°C for 16 hours. The recombinants thus obtained were used for transformation. The transformation was

carried out by electroporation as follows. *E coli* NM522 was cultured on LB medium for 14 to 15 hours. The resulting culture was inoculated on 1L LB so that initial absorbency became 0.07 to 0.1 at 600 nm, and then cultivation was allowed until the absorbency reached 0.8. The cells were centrifuged and suspended in 1L of HEPES [N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic acid)] buffer solution. The cells were again centrifuged and suspended in 500 ml of cold sterile deionized distilled water. The cells were again centrifuged and suspended in 20 ml of 10% glycerol solution. The cells were again centrifuged and suspended in 2 to 3 ml of 10% glycerol solution so that the cell concentration was adjusted to  $2-4 \times [1,010]10^{10}/\text{ml}$ . The cell suspension was rapidly frozen and stored at  $-70^{\circ}\text{C}$ . The frozen cells could be used for about one month during which time their transformation frequency did not decrease.  $40\mu\text{L}$  of frozen cell suspension was thawed in ice and the restored suspension was mixed with the ligated DNA solution. The mixture was put in a gene pulser cuvette with a diameter of 0.2 cm and the capacitance and strength of electric field was fixed at 25  $\mu\text{F}$  and 12.5 kV/cm, respectively. After a single electric pulse was passed at resistance of 200 to 400  $\Omega$ , 1 ml of SOC medium was immediately added and cultured at  $37^{\circ}\text{C}$  for 1 hour. The culture was streaked on LB-ampicillin agar medium and cultivation was allowed for 24 hours to obtain at least fifty thousand colonies. These colonies were together cultured in LB broth for 2 hours. DNA was purely

isolated using an alkaline lysis and the genomic library was constructed therefrom.--

The table beginning on page 18, line 5, has been amended as follows:

--Table 3. Enzyme Titration

Microorganisms	[Non-enzymatic activities] Specific activity of enzyme (U*/mg of protein)	Culture Titer (U/ml of culture solution)
<i>Pseudomonas stutzeri</i> CJ38	0.1	0.023
<i>E. coli</i> ATCC35467/pUC18	0	0
<i>E. coli</i> ATCC35467/pCJ104	0.26	0.175

\*U- $\mu$ mol trehalose/minutes--

The paragraph beginning on page 18, line 22, has been amended as follows:

--The plasmid pCJ104 was subjected to single, double, and triple-digest procedures using about twenty restriction enzymes, such as AatII, BamHI, BglII, SmaI, EcoRI, EcoRV, KpnI, NcoI, NdeI, PstI, SacI, SacII, SalI, SphI and XhoI. DNA fragments were analyzed by electrophoresis through agarose gel and compared to construct the restriction map.--

The heading beginning on page 18, line 28, has been amended as follows:

--**Example [3]8**--

The table beginning on page 20, line 17, has been amended as follows:

--Table 5

Microorganisms	[Non-enzymatic activities] Specific activity of enzyme (U/mg of protein)	Culture Titer of 5 L Fermenter (U/ml of culture)
<i>E. coli</i> ATCC35467/pCJ121	0.43	-
<i>E. coli</i> ATCC35467/pCJ122	4.95	30
<i>E. coli</i> ATCC35467/pCJ123	0	-

--

In the Claims:

The claims have been amended as follows:

3. (Amended) A recombinant plasmid containing the trehalose synthase gene of claim [1] 2.

4. (Amended) The recombinant plasmid according to claim [1] 3 which is recombinant plasmid pCJ122.

5. (Amended) A transformed *E. coli* with the recombinant plasmid of claim [1] 3.



BOX SEQUENCE

PATENT

1599-0206P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: Lee, Se Yong et al. Conf.: 6757  
Appl. No.: 09/937,215 Group: UNASSIGNED  
Filed: September 24, 2001 Examiner: UNASSIGNED  
For: TREHALOSE SYNTHASE PROTEIN, GENE,  
PLASMIDS, MICROORGANISMS, AND A PROCESS  
FOR PRODUCING TREHALOSE

AMENDMENT

Assistant Commissioner for Patents  
Washington, DC 20231

February 6, 2002

Sir:

In response to the Notification of Missing Requirements mailed December 6, 2001, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

In the Specification:

Please replace Table 1 beginning on page 11, line 14, with the following amended Table 1:

Table 1. N-terminal Sequences of Trehalose Synthase Proteins

Source of Trehalose Synthase		N-terminal Sequence
Known Microbes	<i>Thermus aquaticus</i> ATCC 33923	M-D-P-L-W-Y-K-D-A-V-I-Y-Q- (SEQ ID NO: 3)
	<i>Pimelobacter sp.</i> R48	S-T-V-L-G-E-E-P-E-W- F-R-T-A-V-F- Y-E- (SEQ ID NO: 4)
	<i>Pseudomonas putida</i> H262	G-K-W-P-R-P-A-A-F-I-D- (SEQ ID NO: 5)
Transformed <i>E. coli</i> of the Present Invention		S-I-P-D-N-T-Y-I-E-W-L-V- (SEQ ID NO: 6)

Please delete the Sequence Listing of record. Please insert the Substitute Sequence Listing enclosed herewith immediately after the abstract.

#### REMARKS

Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a disk copy of the Substitute Sequence Listing. The disk copy

of the Sequence Listing, file "1599-0206P.ST25", is identical to the paper copy, except that it lacks formatting.


The amendments made to the Specification are intended to reference each amino acid sequence by a unique SEQ ID NO. No new matter is introduced by these amendments.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By

  
Joseph A. Kolasch, #22,463

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

JAK/BCF  
1599-0206P

Attachments:      Disk Copy of Sequence Listing  
                     Paper Copy of Sequence Listing  
                     Copy of Notice to Comply  
                     Version with markings

(Rev. 03/27/01)

1599-0206P-ST25

VERSION WITH MARKINGS TO SHOW CHANGES MADE

(Material being added is shown as bold and underlined. No material is being deleted)

Table 1 beginning on page 11, line 14:

Source of Trehalose Synthase		N-terminal Sequence
Known Microbes	<i>Thermus aquaticus</i> ATCC 33923	M-D-P-L-W-Y-K-D-A-V-I-Y-Q- <u>(SEQ ID NO: 3)</u>
	<i>Pimelobacter sp.</i> R48	S-T-V-L-G-E-E-P-E-W- F-R-T-A-V-F- Y-E- <u>(SEQ ID NO: 4)</u>
	<i>Pseudomonas putida</i> H262	G-K-W-P-R-P-A-A-F-I-D- <u>(SEQ ID NO: 5)</u>
Transformed <i>E. coli</i> of the Present Invention		S-I-P-D-N-T-Y-I-E-W-L-V- <u>(SEQ ID NO: 6)</u>

TREHALOSE SYNTHASE PROTEIN, GENE,  
PLASMIDS, MICROORGANISMS, AND A  
PROCESS FOR PRODUCING TREHALOSE

## BACKGROUND OF THE INVENTION

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## FIELD OF THE INVENTION

The present invention relates to a trehalose-producing microorganism and a process for producing trehalose. It also relates to a novel trehalose synthase protein,  
10 a trehalose synthase gene, recombinant plasmids carrying said trehalose synthase gene, and transformed microorganisms with said recombinant plasmids.

## DESCRIPTION OF THE PRIOR ART

15 Trehalose is a non-reducing disaccharide, two saccharides of which are linked by  $\alpha$ -1,1 bond:  $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside. It has wide application in medicines, foods, and cosmetics. However, its utilization has been greatly restricted because its production to date has been inefficient and expensive.

20 Japanese Laid-open Patent Nos. Hei5-91890 and Hei6-145186 disclose methods for extracting trehalose from yeasts. There are several methods for isolating trehalose from fermented microorganism cultures, such as *Arthrobacter* (T. Suzuki, Agric. Biol. Chem., 33(2), 1969), *Nocardia* (Japanese Laid-open Patent No. Sho50-154485), *Micrococcus* (Japanese Laid-open Patent No. Hei6-319578), amino acid-fermenting yeast, *Brevibacterium* (Japanese Laid-open Patent No. Hei5-211882), and  
25 yeast (Yoshikwa, etc., Biosci. Biotech. Biochem., 1994, 58, 1226-12300). Additionally, a method for producing trehalose by using recombinant plants including bacterial genes capable of converting glucose into trehalose is described in M. Scher, Food Processing, April, 95-96, 1993. Japanese Laid-open Patent No. 83-216695  
30 discloses a method for converting maltose into trehalose by using maltose phosphorylase and trehalose phosphorylase. However, these methods are not effective because their procedures are complicated and their yields are low.

Several enzymatic methods have been published recently. Japanese Laid-open Patent No. Hei7-143876 and EPO 628630 A2 discloses a two-step enzymatic conversion method in which starch is converted into trehalose by maltooligosyl trehalose synthase and maltooligosyl trehalose trehalohydrolase. Japanese Laid-open Patent No. Hei7-170977 and Korean Laid-open Patent No. 95-3444 disclose one-step enzymatic conversion methods in which maltose is directly converted into trehalose by trehalose synthase. However, there is still a need to increase the titer of the trehalose synthase enzyme so that production of trehalose from maltose becomes more efficient in yield and cost.

We have invested much effort over the last several years in isolating microorganisms able to convert maltose into trehalose from soil. We have successfully screened a novel strain which highly expresses trehalose and, unexpectedly, generates no byproducts, unlike all known microorganisms. Its morphological and physiological characteristics identify it as a novel *Pseudomonas stutzeri* strain. This strain has been designated as *Pseudomonas stutzeri* CJ38.

We isolated a trehalose synthase gene from chromosomes of *Pseudomonas stutzeri* CJ38 and determined its nucleotide sequence by cloning it into known vector pUC18 with restriction enzyme *Sau3AI*. In addition, we isolated a trehalose synthase protein from *Pseudomonas stutzeri* CJ38 and determined its amino acid sequence using standard methods. It was found that these sequences are apparently different from the sequences of the trehalose synthase gene and all proteins known hitherto. This invention was achieved by constructing recombinant plasmids carrying the trehalose synthase gene so that the trehalose synthase enzyme encoded in said gene can be expressed in large amounts.

## SUMMARY OF THE INVENTION

The present invention provides a novel microorganism, *Pseudomonas stutzeri* CJ38, that produces trehalose from maltose. This strain was deposited at the Korea Culture Center of Microorganisms, Seoul, Korea, as the accession number KCCM 10150 on February 12, 1999 under the Budapest Treaty. This strain is very valuable

as it does not generate byproducts such as glucose when converts maltose into trehalose.

The present invention also provides a novel trehalose synthase protein with the following amino acid sequence:

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Met Ser Ile Pro Asp Asn Thr Tyr Ile Glu Trp Leu Val Ser Gln

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Ser Met Leu His Ala Ala Arg Glu Arg Ser Arg His Tyr Ala Gly

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Gln Ala Arg Leu Trp Gln Arg Pro Try Ala Gln Ala Arg Pro Arg

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Asp Ala Ser Ala Ile Ala Ser Val Trp Phe Thr Ala Tyr Pro Ala

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Ala Ile Ile Thr Pro Glu Gly Gly Thr Val Leu Glu Ala Leu Gly

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70

75

Asp Asp Arg Leu Trp Ser Ala Leu Ser Glu Leu Gly Val Gln Gly

80

85

90

Ile His Asn Gly Pro Met Lys Arg Ser Gly Gly Leu Arg Gly Arg

95

100

105

20

Glu Phe Thr Pro Thr Ile Asp Gly Asn Phe Asp Arg Ile Ser Phe

110

115

120

Asp Ile Asp Pro Ser Leu Gly Thr Glu Glu Gln Met Leu Gln Leu

125

130

135

Ser Arg Val Ala Ala Ala His Asn Ala Ile Val Ile Asp Asp Ile

25

140

145

150

Val Pro Ala His Thr Gly Lys Gly Ala Asp Phe Arg Leu Ala Glu

155

160

165

Met Ala Tyr Gly Asp Tyr Pro Gly Leu Tyr His Met Val Glu Ile

170

175

180

30

Arg Glu Glu Asp Trp Glu Leu Leu Pro Glu Val Pro Ala Gly Arg

185

190

195

Asp Ser Val Asn Leu Leu Pro Pro Val Val Asp Arg Leu Lys Glu

200

205

210

Lys His Tyr Ile Val Gly Gln Leu Gln Arg Val Ile Phe Phe Glu  
 215 220 225  
 Pro Gly Ile Lys Asp Thr Asp Trp Ser Val Thr Gly Glu Val Thr  
 230 235 240  
 5 Gly Val Asp Gly Lys Val Arg Arg Trp Val Tyr Leu His Tyr Phe  
 245 250 255  
 Lys Glu Gly Gln Pro Ser Leu Asn Trp Leu Asp Pro Thr Phe Ala  
 260 265 270  
 10 Ala Gln Gln Leu Ile Ile Gly Asp Ala Leu His Ala Ile Asp Val  
 275 280 285  
 Thr Gly Ala Arg Val Leu Arg Leu Asp Ala Asn Gly Phe Leu Gly  
 290 295 300  
 Val Glu Arg Arg Ala Glu Gly Thr Ala Trp Ser Glu Gly His Pro  
 305 310 315  
 15 Leu Ser Val Thr Gly Asn Gln Leu Leu Ala Gly Ala Ile Arg Lys  
 320 325 330  
 Ala Gly Gly Phe Ser Phe Gln Glu Leu Asn Leu Thr Ile Asp Asp  
 335 340 345  
 20 Ile Ala Ala Met Ser His Gly Gly Ala Asp Leu Ser Tyr Asp Phe  
 350 355 360  
 Ile Thr Arg Pro Ala Tyr His His Ala Leu Leu Thr Gly Asp Thr  
 365 370 375  
 Glu Phe Leu Arg Met Met Leu Arg Glu Val His Ala Phe Gly Ile  
 380 385 390  
 25 Asp Pro Ala Ser Leu Ile His Ala Leu Gln Asn His Asp Glu Leu  
 395 400 405  
 Thr Leu Glu Leu Val His Phe Trp Thr Leu His Ala Tyr Asp His  
 410 415 420  
 Tyr His Tyr Lys Gly Gln Thr Leu Pro Gly Gly His Leu Arg Glu  
 425 430 435  
 30 His Ile Arg Glu Glu Met Tyr Glu Arg Leu Thr Gly Glu His Ala  
 440 445 450

Pro Tyr Asn Leu Lys Phe Val Thr Asn Gly Val Ser Cys Thr Thr  
455 460 465

Ala Ser Val Ile Ala Ala Ala Leu Asn Ile Arg Asp Leu Asp Ala  
470 475 480

5 Ile Gly Pro Ala Glu Val Glu Gln Ile Gln Arg Leu His Ile Leu  
485 490 495

Leu Val Met Phe Asn Ala Met Gln Pro Gly Val Phe Ala Leu Ser  
500 505 510

10 Gly Trp Asp Leu Val Gly Ala Leu Pro Leu Ala Pro Glu Gln Val  
515 520 525

Glu His Leu Met Gly Asp Gly Asp Thr Arg Trp Ile Asn Arg Gly  
530 535 540

Gly Tyr Asp Leu Ala Asp Leu Ala Pro Glu Ala Ser Val Ser Ala  
545 550 555

15 Glu Gly Leu Pro Lys Ala Arg Ser Leu Tyr Gly Ser Leu Ala Glu  
560 565 570

Gln Leu Gln Arg Pro Gly Ser Phe Ala Cys Gln Leu Lys Arg Ile  
575 580 585

20 Leu Ser Val Arg Gln Ala Tyr Asp Ile Ala Ala Ser Lys Gln Ile  
590 595 600

Leu Ile Pro Asp Val Gln Ala Pro Gly Leu Leu Val Met Val His  
605 610 615

Glu Leu Pro Ala Gly Lys Gly Val Gln Leu Thr Ala Leu Asn Phe  
620 625 630

25 Ser Ala Glu Pro Val Ser Glu Thr Ile Cys Leu Pro Gly Val Ala  
635 640 645

Pro Gly Pro Val Val Asp Ile Ile His Glu Ser Val Glu Gly Asp  
650 655 660

30 Leu Thr Asp Asn Cys Glu Leu Gln Ile Asn Leu Asp Pro Tyr Glu  
665 670 675

Gly Leu Ala Leu Arg Val Val Ser Ala Ala Pro Pro Val Ile  
680 685

In addition, the present invention provides a novel trehalose synthase gene with the following nucleotide sequence:

	GATCGCTGGC	GTACTGCAGG	TAGAGCAGGC	GCATCGGCCC	CCAGGGCGCA	TCGGCCGGCT	60
	CCGCTGTGCC	CTGCTGGTTC	ATGAAGCGGA	CGAAGCGGCC	ATCGCGGAAC	CGTGGACGCC	120
5	ATTCGGGGCT	GTCCGGGTCG	CGGCTGTTCG	TGAGCGTGCG	CCACAGGTCG	CTGCGAAACG	180
	GCGGACCGCT	CCAAAGCGCG	CCGTGGATGG	GATCGCCGAG	CAGTTCGTGC	AGTCCCAAG	240
	AACGTTGCGA	ATGCAGCGCG	CCGAGGCTCA	GGCCATGCAG	ATACAGGCGC	GGTCGGCGTT	300
	CGGCCGGCAG	TTCGGTCCAG	TAGCCATAGA	TCTCGGCGAA	TAGCGCGCGG	GCCACGTTCG	360
	GGCCGTAGTC	GGCCTCCACC	AGCAGCGCCA	GCGGGCTGTT	CAGATAGGAG	TACTGCAACG	420
10	CCACGCTGGC	GATATCGCCG	TGGTGCAGGT	ATTCCACTGC	GTTTCATCGC	GCCGGGTCGA	480
	TCCAGCCGGT	ACCGGTGGGC	GTCACCAGCA	CCAGCACCGA	TCGCTCGAAG	GCGCCGCTGC	540
	GCTGCAGCTC	GCGCAAGGCC	AGACGCGCCC	GCTGGCGCGG	GGTCTCTGCC	GCGCGCAGAC	600
	CGACGTAGAC	GCGAATCGGC	TCGAGCGCCG	AGCGGCCGCT	CAAGACGCTG	ATATCCGCCG	660
	CCGACGGGCC	GGAGCCGATG	AACTCGCGGC	CGGTGCGGCC	CAGCTCCTCC	CAGCGCAGCA	720
15	ACGAGGCCCG	GCTGCCGCTT	TTCAGCGGCG	AGGCCGGTGG	GCCTCTCTCC	GGTTCGATCA	780
	GGGCGTCGTA	CTGCGCGAAG	GATGCGTCCA	GCATGCGCAG	TGCCCCGCGC	GCCAGCACAT	840
	CGCTGAGCAG	CGACCAGAAC	AGCGCCAGCG	CCACCAGCAC	GCCGATCAGC	TTGGCCAGGC	900
	GCCGTGGCAG	CACGCGGTCT	GCGTGCCGCG	AGACGAAGCG	CGACACCAGC	CGATACAGAC	960
	GCGCCAGCGT	CAGCAGGATG	AGAAAGTCTG	CCAGCGCGGT	GAGAATGACT	TCGAGCAGGT	1020
20	GCGCACTGCT	CACCGGCGGC	ATGCCCATCA	GCGCGCGTAC	CGCGTTCTGC	CAGCCGGCGA	1080
	CCTGGCTGAG	GAAATACCCG	GCCAGCAGCA	GGCAGCCGAC	CGCGATCAGC	AGATTGACCC	1140
	GCTCGCGCTG	CCAGCCTGGG	CGCTCCGGCA	GTTCCAGATA	GCGCCACAGC	CAGCGCCAGA	1200
	ACACGCCGAG	GCCATAGCCC	ACCGCCAGCG	CCGCGCCGGC	CAGCACGCCC	TGGCTCAGCG	1260
	TCGAGCGCGG	CAGCAGCGAT	GGCGTCAGCG	CCGCGCAGAA	GAACAGCGTG	CCCAGCAGCA	1320
25	GGCCGAAACC	GGACAGCGAG	CGCCAGATAT	AGAGGACGGG	CAGGTGCAGC	ATGAAGATCT	1380
	CCGCGGTCTG	GTGACGGCGT	CGCGCCTCGG	CATATCGAGG	CGTGTCCGGT	CGTGCGGTTC	1440
	CCGTGATGGT	CCGAGCAGG	CCAATCCGAT	GCAACGATGG	CCGAGCGGCC	GACTCAAACG	1500
	TCTACATTTC	CCTAGTGCTG	CCGGAACCGA	TCGCCG			1536
	ATG AGC ATC	CCA GAC AAC	ACC TAT ATC	GAA TGG CTG	GTC AGC CAG	TCC	1584
30	ATG CTG CAT	GCG GCC CGC	GAG CGG TCG	CGT CAT TAC	GCC GGC CAG	GCG	1632
	CGT CTC TGG	CAG CGG CCT	TAT GCC CAG	GCC CGC CCG	CGC GAT GCC	AGC	1680
	GCC ATC GCC	TCG GTG TGG	TTC ACC GCC	TAT CCG GCG	GCC ATC ATC	ACG	1728
	CCG GAA GGC	GGC ACG GTA	CTC GAG GCC	CTC GGC GAC	GAC CGC CTC	TGG	1776

	AGT GCG CTC TCC GAA CTC GGC GTG CAG GGC ATC CAC AAC GGG CCG ATG	1824
	AAG CGT TCC GGT GGC CTG CGC GGA CGC GAG TTC ACC CCG ACC ATC GAC	1872
	GGC AAC TTC GAC CGC ATC AGC TTC GAT ATC GAC CCG AGC CTG GGG ACC	1920
	GAG GAG CAG ATG CTG CAG CTC AGC CGG GTG GCC GCG GCG CAC AAC GCC	1968
5	ATC GTC ATC GAC GAC ATC GTG CCG GCA CAC ACC GGC AAG GGT GCC GAC	2016
	TTC CGC CTC GCG GAA ATG GCC TAT GGC GAC TAC CCC GGG CTG TAC CAC	2064
	ATG GTG GAA ATC CGC GAG GAG GAC TGG GAG CTG CTG CCC GAG GTG CCG	2112
	GCC GGG CGT GAT TCG GTC AAC CTG CTG CCG CCG GTG GTC GAC CGG CTC	2160
	AAG GAA AAG CAC TAC ATC GTC GGC CAG CTG CAG CGG GTG ATC TTC TTC	2208
10	GAG CCG GGC ATC AAG GAC ACC GAC TGG AGC GTC ACC GGC GAG GTC ACC	2256
	GGG GTC GAC GGC AAG GTG CGT CGC TGG GTC TAT CTG CAC TAC TTC AAG	2304
	GAG GGC CAG CCG TCG CTG AAC TGG CTC GAC CCG ACC TTC GCC GCG CAG	2352
	CAG CTG ATC ATC GGC GAT GCG CTG CAC GCC ATC GAC GTC ACC GGC GCC	2400
	CGG GTG CTG CGC CTG GAC GCC AAC GGC TTC CTC GGC GTG GAA CGG CGC	2448
15	GCC GAG GGC ACG GCC TGG TCG GAG GGC CAC CCG CTG TCC GTC ACC GGC	2496
	AAC CAG CTG CTC GCC GGG GCG ATC CGC AAG GCC GGC GGC TTC AGC TTC	2544
	CAG GAG CTG AAC CTG ACC ATC GAT GAC ATC GCC GCC ATG TCC CAC GGC	2592
	GGG GCC GAT CTG TCC TAC GAC TTC ATC ACC CGC CCG GCC TAT CAC CAT	2640
	GCG TTG CTC ACC GGC GAT ACC GAA TTC CTG CGC ATG ATG CTG CGC GAA	2688
20	GTG CAC GCC TTC GGC ATC GAC CCG GCG TCA CTG ATC CAT GCG CTG CAG	2736
	AAC CAT GAC GAG TTG ACC CTG GAG CTG GTG CAC TTC TGG ACG CTG CAC	2784
	GCC TAC GAC CAT TAC CAC TAC AAG GGC CAG ACC CTG CCC GGC GGC CAC	2832
	CTG CGC GAA CAT ATC CGC GAG GAA ATG TAC GAG CGG CTG ACC GGC GAA	2880
	CAC GCG CCG TAC AAC CTC AAG TTC GTC ACC AAC GGG GTG TCC TGC ACC	2928
25	ACC GCC AGC GTG ATC GCC GCG GCG CTT AAC ATC CGT GAT CTG GAC GCC	2976
	ATC GGC CCG GCC GAG GTG GAG CAG ATC CAG CGT CTG CAT ATC CTG CTG	3024
	GTG ATG TTC AAT GCC ATG CAG CCC GGC GTG TTC GCC CTC TCC GGC TGG	3072
	GAT CTG GTC GGC GCC CTG CCG CTG GCG CCC GAG CAG GTC GAG CAC CTG	3120
	ATG GGC GAT GGC GAT ACC CGC TGG ATC AAT CGC GGC GGC TAT GAC CTC	3168
30	GCC GAT CTG GCG CCG GAG GCG TCG GTC TCC GCC GAA GGC CTG CCC AAG	3216
	GCC CGC TCG CTG TAC GGC AGC CTG GCC GAG CAG CTG CAG CGG CCA GGC	3264
	TCC TTC GCC TGC CAG CTC AAG CGC ATC CTC AGC GTG CGC CAG GCC TAC	3312
	GAC ATC GCT GCC AGC AAG CAG ATC CTG ATT CCG GAT GTG CAG GCG CCG	3360

	GGA CTC CTG GTG ATG GTC CAC GAG CTG CCT GCC GGC AAG GGC GTG CAG	3408
	CTC ACG GCA CTG AAC TTC AGC GCC GAG CCG GTC AGC GAG ACC ATC TGC	3456
	CTG CCC GGC GTG GCG CCC GGC CCG GTG GTG GAC ATC ATT CAC GAG AGT	3504
	GTG GAG GGC GAC CTC ACC GAC AAC TGC GAG CTG CAG ATC AAC CTC GAC	3552
5	CCG TAC GAG GGG CTT GCC CTG CGT GTG GTG AGC GCC GCG CCG CCG GTG	3600
	ATC TGA GCGC	3610
	CCTCTTCGCG CGCCCCGGGT CCGCCGCTAT AGTGCGCAGC GCCTGGGGCG CGCATTGCC	3670
	TCGCCGTCGA GACCAGCCCG TGTCGTTTAC TTCGCTTTTC CGCCTTGCGC TGCTGCCGCT	3730
	GGCGCTGCTT GCCGCACCCG TCTGGGCGCA GACCGCTGC CCGCCCGGCC AGCAGCCGAT	3790
10	CTGCCTGAGC GGCAGCTGCC TCTGCGTGCC GGCGCGGCC AGCGATCCAC AGGCGGTCTA	3850
	CGACCGCGTG CAGCGTATGG CTACGCTGGC CCTGCAGAAC TGGATCCAGC AGTCGCGCGA	3910
	CCGCTGATG GCCGGCGGCG TCGAGCCGAT ACCGCTGCAC ATCCGCTCGC AGCTCGAGCC	3970
	GTATTTGAT CTTGCCGTGC TGGAGAGTGC GCGGTACCGC GTCGGCGACG AGGTGGTGCT	4030
	GACTGCCGGC AACACCCTGC TGCGCAACCC GGACGTCAAT GCCGTGACCC TGATCGACGT	4090
15	CATCGTCTTC CGCCACGAGG AGGATGCCCC GGACAACGTC GCGCTCTGGG CCCATGAGCT	4150
	CAAGCACGTC GAGCAATATC TGGACTGGGG CGTCGCCGAG TTCGCCCGC GCTATACGCA	4210
	GGATTTCCGT GCCGTGGAGC GCCCGGCCTA TGGCTGGAG CGTGAGGTGG AAGAGGCCCT	4270
	GCGCGAGACG CAGACGCGGC GCTGAGCGAG CTGATCGGTG CTGCTGCCCG CACTGGGCTG	4330
	AAGCCACCA ATGACGCCGG CGAAAACGAA AAACCCCGCC GAGGCGGGGT TTCTGACGCG	4390
20	GGTTGTGCGG TCAGCTCAGA ACGCCGGGAC CACGGCGCCC TTGTACTTTT CCTCGATGAA	4450
	CTGGCTACT TGCTCGCTGT GCAGCGCGGC AGCCAGTTTC TGCATGGCAT CGCTGTCCTT	4510
	GTTGTCCGGA CGGGCGACCA GAATGTTTAC GTATGGCGAG TCGCTGCCCT CGATCACCAG	4570
	GGCGTCCTGG GTCGGGTTC GCTTGGCTTC CAGCGGTAG TTGGTGTTGA TCAGCGCCAG	4630
	GTCGACCTGG GTCAGCACGC GCGGCAGAGT CGCGGCTTCC AGTTCGCGGA TCTTGATCTT	4690
25	CTTCGGGTTC TCGGCGATGT CTTCGGCGTG GCGGTGATGC CGGCGCCGTC CTTCAGACCG	4750
	ATC	4753

The present invention also provides a recombinant plasmid containing the trehalose synthase gene with the above nucleotide sequence. In a preferred embodiment, the present invention provides a recombinant plasmid pCJ104 in which the 4.7 kb *Sau3AI* DNA fragment of the trehalose synthase gene of the present invention is cloned into vector plasmid pUC18. This allow for the efficient and high

expression of the trehalose synthase gene. In a more preferred embodiment, the present invention provides a recombinant plasmid pCJ122 in which the 2.5 kb *Bam*HI-*Bgl*II DNA fragment of the trehalose synthase gene of the present invention is included in a vector plasmid pUC18, allowing for a higher expression of the trehalose synthase gene.

5

The present invention provides a transformed *E. coli* with a recombinant plasmid containing the trehalose synthase gene with the above nucleotide sequence. In a preferred embodiment, the present invention provides a transformed *E. coli* with a recombinant plasmid pCJ104, allowing for production of high levels of the trehalose synthase protein. In a more preferable embodiment, the present invention provides a transformed *E. coli* with the recombinant plasmid pCJ122, allowing for production of even higher levels of the trehalose synthase protein.

10

The present invention provides a process for producing trehalose which comprises reacting the trehalose synthase protein with the above amino acid sequence with maltose solution to obtain trehalose.

15

The present invention provides a process for producing trehalose which comprises crushing a transformed *E. coli* with a recombinant plasmid containing the trehalose synthase gene with the above nucleotide sequence and reacting the crushed cells with maltose solution to obtain trehalose. In a preferred embodiment, the present invention provides a process for producing trehalose which comprises crushing a transformed *E. coli* with plasmid pCJ104, centrifuging the crushed cells and reacting the resulting supernatant with maltose solution to obtain trehalose. In a more preferable embodiment, the present invention provides a process for producing trehalose which comprises crushing a transformed *E. coli* with plasmid pCJ122, centrifuging the crushed cells and reacting the resulting supernatant with maltose solution to obtain trehalose.

20

25

### 30 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an analysis of saccharides by thin-layer chromatography to

which a reaction solution containing sonicated liquid from *Pseudomonas stutzeri* CJ38 and maltose solution was subjected. The symbols G, M and T indicate glucose, maltose and trehalose, respectively.

Figure 2 shows an analysis of saccharides by gas chromatography to which a reaction solution (A) containing sonicated liquid from *Pseudomonas stutzeri* CJ38 and maltose solution and a standard trehalose specimen (B) were subjected. The symbol Tre indicate trehalose.

Figure 3 shows an analysis of saccharides by high performance liquid chromatography to which a standard trehalose specimen (A), and specimens (B) and (C) were subjected. Specimen (B) was obtained just after a solution containing sonicated liquid from *Pseudomonas stutzeri* CJ38 and maltose solution was reacted completely. Specimen (C) was obtained by adding trehalase to a reaction solution containing sonicated liquid from *Pseudomonas stutzeri* CJ38 and maltose solution after completion of their reaction. The symbols Tre, Mal and Glu indicate trehalose, maltose and glucose, respectively.

Figure 4 shows a construction map of a recombinant plasmid pCJ104 including a trehalose synthase gene of the present invention.

Figure 5 shows a restriction map of a 4.7 kb *Sau3AI* fragment within a recombinant plasmid pCJ104 of the present invention.

Figure 6 shows a construction map of recombinant plasmids pCJ121 and pCJ122 of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

A microorganism which produces trehalose from maltose by trehalose synthase was isolated from soil and identified as having the morphological and physiological characteristics of *Pseudomonas stutzeri*. *Pseudomonas stutzeri* has not been reported to convert maltose into trehalose. Therefore, the microorganism isolated

by us can be recognized as a novel *Pseudomonas stutzeri* strain and has been designated as *Pseudomonas stutzeri* CJ38.

We constructed the restriction map of a recombinant plasmid pCJ104 of the present invention using various restriction enzymes. Two trehalose synthase gene sequences are known (Biochim. Biophys. Acta 1996, 1290, 1-3 and Biochim. Biophys. Acta 1997, 1334, 28-32). The comparison of the present and known restriction maps revealed that pCJ104 represents different patterns from those known.

Trehalose synthase proteins from known microorganisms have shown similarities in their N-terminus. However, it was found that the N-terminal sequence of the trehalose synthase protein of the present invention is not identical with those of known trehalose synthase proteins. The results are shown in Table 1 below.

Table 1. N-terminal Sequences of Trehalose Synthase Proteins

	Source of Trehalose Synthase	N-terminal Sequence
Known Microbes	<i>Thermus aquaticus</i> ATCC 33923	M-D-P-L-W-Y-K-D-A-V-I-Y-Q-
	<i>Pimelobacter sp.</i> R48	S-T-V-L-G-E-E-P-E-W-F-R-T-A-V-F-Y-E-
	<i>Pseudomonas putida</i> H262	G-K-W-P-R-P-A-A-F-I-D-
	Transformed <i>E. coli</i> of the Present Invention	S-I-P-D-N-T-Y-I-E-W-L-V-

The nucleotide sequence of 4.7 kb *Sau3AI* fragment within a recombinant plasmid pCJ104 of the present invention and the amino acid sequence of a trehalose synthase protein expressed therefrom were determined (SEQ ID NO: 1).

In addition, the intact sequence of a trehalose synthase protein of the present invention was compared to those of the trehalose synthase proteins disclosed in Biochim. Biophys. Acta 1996, 1290, 1-3 and Biochim. Biophys. Acta 1997, 1334, 28-

32. The comparison revealed that there are no similarities between them.

The enzymatic conversion reaction was carried out using crushed *E. coli* transformants including recombinant plasmids pCJ104 or pCJ122. As a result, the titer of trehalose synthase enzyme from the crushed cells of the present invention was considerably higher than that from the wild type *Pseudomonas stutzeri* CJ38.

The properties and availabilities of the plasmids and microorganisms used in and prepared by the present invention are shown in Table 2 below.

Table 2

Microbes and Plasmids	Properties	Availability
<i>Pseudomonas stutzeri</i> CJ38	Wild type strain producing the trehalose synthase enzyme of the present invention	KFCC-10985
<i>E. coli</i> NM522	hsd $\Delta$ 5, $\Delta$ (lac <sup>+</sup> pro) [F <sup>+</sup> , Pro <sup>+</sup> , lacI <sup>q</sup> Z $\Delta$ M15]	Amersham
<i>E. coli</i> ATCC35467	[malP,Q::Tn5 ompBCS1 F <sup>-</sup> araD139 $\Delta$ (argF <sup>-</sup> lac) 205U169 rpsL150 relA1 flbB5301 deoC1 ptsF25]	ATCC
pCJ104	pUC18 containing 4.7 kb Sau3AI DNA fragment (trehalose synthase gene), Ap <sup>r</sup>	Constructed
pCJ121	pUC18 containing 3.35 kb KpnI DNA fragment (trehalose synthase gene), Ap <sup>r</sup>	Constructed (Control)
pCJ122	pUC18 containing 2.5 kb BamHI-BglII DNA fragment (trehalose synthase gene), Ap <sup>r</sup>	Constructed
pCJ123	pUC18 containing 1.2 kb BamHI-EcoRI DNA fragment	Constructed (Control)
pUC18 and pUC19	Ap <sup>r</sup> , 2.7 kb	New England Biolabs

Nutrient medium (0.3% broth, 0.5% peptone, pH 6.8) and LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) were used for cultivation of *Pseudomonas stutzeri* and *E. coli*, respectively. For the culture of cells transformed by electroporation, SOC medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>, 20 mM glucose) was used. MacConkey agar medium (4% bacto MacConkey agar base, 2.0% maltose, pH 7.0) was used in cloning the trehalose synthase gene. Ampicillin was added in a concentration of 50 mg/L. Gene Pulser (Bio-Rad) was used in transformation of *E. coli* by electroporation. The genetic manipulation used in the present invention was carried out in accordance with procedures described in *Molecular Cloning, Laboratory Manual*, 2<sup>nd</sup> ed., Sambrook, J., E.F. Frisch and T. Maniatis and *Guide to Molecular Cloning Techniques, Methods in Enzymol.* Vol. 152, Berger, S.L., A.R. Kimme.

The enzymatic reaction is conducted at pH 6.0 to 7.0, preferably pH 7.0 to 10, and at temperatures of 4°C to 45°C, preferably 20°C to 40°C. Maltose can be used as a substrate in a concentration of less than 50%. The trehalose synthase enzyme can be used in a pure form or in crushed cells.

The following examples illustrate the present invention. From the foregoing description and the following examples, it is believed that those skilled in the art would be able to carry out the invention completely.

### Example 1

#### Screening of Microorganism

A platinum loop of microorganisms, isolated from soil, was inoculated in a 500 ml Erlenmeyer flask containing 50 ml of LB culture solution (0.5% of yeast extract, 1.0% of bactotrypton, 0.5% of salt) and cultured at 28°C for 2 days. The culture was centrifuged at 4°C, 8,000 rpm, for 5 minutes. The cells were collected and washed with physiological saline. The washed cells were suspended in 10 ml of phosphate buffer solution (10 mM, pH 7.0). The cells were crushed by an ultrasonicator and the crushed cells were centrifuged at 4°C, 1,200 rpm, for 20 minutes and the supernatant was used as a crude enzymatic solution. The

concentration of the protein in the crude enzymatic solution was determined by the Bredford method. 100 µg of protein was mixed with 20 µl of 100 mM maltose and 10 µl of 100 mM phosphate buffer solution (pH 7.0). Distilled water was added to the mixture until the total volume reached 100 µl and the reaction occurred at 30°C for 20 hours. The saccharides present in the reaction solution were analyzed by TLC, HPLC, and GC.

### **Example 2**

#### **Analysis of Trehalose by Thin-layer Chromatography (Figure 1)**

After the reaction was completed, 5 µl of the reaction solution were spotted on Kieselgel 60 TLC (Merck, Germany) and placed in a vessel containing a solvent system of n-butanol-pyridine-water (7:3:1) to develop the specimens. It was sprayed with a solution of 20% sulfuric acid in methyl alcohol and dried at 110°C for 10 minutes. The saccharides in the specimens were thus specified. Among at least 1,000 soil microorganisms investigated, two were confirmed to have the ability to convert maltose into trehalose. Figure 1 shows that trehalose did not exist in the specimens prior to the reaction but, after completion of the reaction, saccharides were detected at the site of a standard trehalose specimen.

### **Example 3**

#### **Analysis of Trehalose by Gas Chromatography (Figure 2)**

After completion of the reaction, 10 µl of the reaction solution was dried by a reduced pressure dryer. The dried product was dissolved in 20 µl of dimethylformamide and the resulting solution was mixed with the same volume of bis(trimethyl)trifluoroacetamide to form trimethylsilane derivatives. One µl of aliquot was used in GC analysis. As shown in Figure 2, the peak of the reaction solution was observed to occur at the same time as with a standard trehalose specimen.

### **Example 4**

#### **Analysis of Trehalose by High Performance Liquid Chromatography (Figure 3)**

After the reaction was completed, half of the reaction solution was mixed with the same volume of phenol to remove proteins. The specimen solution thus obtained was used in the HPLC analysis. The peak of the specimen was observed to occur at the same time as with a standard trehalose specimen. The remaining half of the reaction solution was heated to 100°C for 10 minutes to terminate enzyme activity.

5 It was reacted at 37°C for 10 minutes with trehalase (Sigma) which specifically acts on  $\alpha$ -1,1-trehalose. After completion of the reaction, the solution was mixed with the same volume of phenol solution to remove proteins. The solution obtained thus was subjected to HPLC, and as a result the peak disappeared at the same time as with a standard trehalose.

10

### Example 5

#### Identification of Microorganism Capable of Converting Maltose into Trehalose

15 The soil microorganism of the present invention was observed by electron microscope and is characterized by rod shaped bacteria with flagellum. It was also characterized as aerobic by an O/F test and by Gram-negative. The physiological characteristics of the microorganism are summarized in Table 1. These characteristics of the present microorganism were compared to those of microorganisms described in *Bergey's Manual of Systemic Bacteriology*, 1984 and in patent publications, and it

20 was classified as *Pseudomonas stutzeri*, because it is almost identical to that microorganism, physiologically and morphologically.

Table 1

25	DP3 -	OFG +	GC +	ACE -	ESC -	PLI -
	URE -	CIT +	MAL +	TDA -	PXB -	LAC -
	MLT +	MAN +	XYL -	RAF -	SOR -	SUC -
	INO -	ADO -	COU -	H <sub>2</sub> S -	ONP -	RHA -
	ARA -	GLU -	ARG -	LYS -	ORN -	OXI -
	TLA -					

30

### Example 6

#### Cloning of Trehalose Synthase Gene (Figure 4)

(1) Isolation of Chromosomal DNA from *Pseudomonas stutzeri*

*Pseudomonas stutzeri* was grown in a nutrient medium and at an early resting stage, cells were recovered by centrifugation. The recovered cells were washed twice  
5 with TE solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). The washed cells were suspended in 20 mL of STE buffer (20% sucrose, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) and 5 mg/mL of lysozyme and RNase A were added to the suspension. The reaction occurred at 37°C for 2 hours. After the reaction was completed, SDS was added up to a concentration of 1% and the reaction continued  
10 at 37°C for 30 minutes. This solution was reacted with the same volume of phenol for 4 hours and was subjected to centrifugation. 5M NaCl was added to the resulting supernatant until its concentration reached 0.1 M. Using a glass bar, a two-fold volume of anhydrous ethanol was added to obtain chromosomal DNA. The chromosomal DNA was washed with 70% ethanol and dissolved in TE solution for  
15 use in the next experiment.

(2) Preparation of Genomic Library

The pure chromosomal DNAs isolated from *Pseudomonas stutzeri* were  
20 partially digested with restriction enzyme *Sau3AI* at 37°C for 15 to 30 minutes. The restriction enzyme was inactivated with heat and agarose gel electrophoresis was carried out to obtain 3 to 10 kb DNA fragments. As shown in Figure 5, plasmid pUC18 was digested with *Bam*HI and was treated with calf intestinal phosphatase. The cleaved DNAs were mixed with 3 to 10 kb DNA fragments previously obtained  
25 and ligation with T4 DNA ligase was allowed at 15°C for 16 hours. The recombinants thus obtained were used for transformation. The transformation was carried out by electroporation as follows. *E. coli* NM522 was cultured on LB medium for 14 to 15 hours. The resulting culture was inoculated on 1L LB so that initial absorbency became 0.07 to 0.1 at 600 nm, and then cultivation was allowed until the absorbency  
30 reached 0.8. The cells were centrifuged and suspended in 1L of HEPES [N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic acid)] buffer solution. The cells were again centrifuged and suspended in 500 ml of cold sterile deionized distilled water.

The cells were again centrifuged and suspended in 20 ml of 10% glycerol solution. The cells were again centrifuged and suspended in 2 to 3 ml of 10% glycerol solution so that the cell concentration was adjusted to  $2-4 \times 10^{10}$ /ml. The cell suspension was rapidly frozen and stored at  $-70^{\circ}\text{C}$ . The frozen cells could be used for about one month during which time their transformation frequency did not decrease. 40  $\mu\text{L}$  of frozen cell suspension was thawed in ice and the restored suspension was mixed with the ligated DNA solution. The mixture was put in a gene pulser cuvette with a diameter of 0.2 cm and the capacitance and strength of electric field was fixed at 25  $\mu\text{F}$  and 12.5 kV/cm, respectively. After a single electric pulse was passed at resistance of 200 to 400  $\Omega$ , 1 ml of SOC medium was immediately added and cultured at  $37^{\circ}\text{C}$  for 1 hour. The culture was streaked on LB-ampicillin agar medium and cultivation was allowed for 24 hours to obtain at least fifty thousand colonies. These colonies were together cultured in LB broth for 2 hours. DNA was purely isolated using an alkaline lysis and the genomic library was constructed therefrom.

### (3) Cloning of Trehalose Synthase Gene

*E. coli* ATCC35467, which is unable to utilize maltose as a carbon source, was transformed with the genomic library obtained from the above by electroporation. The transformed cells were streaked on a MacConkey-ampicillin agar medium containing 20 g/L of maltose. Once the trehalose synthase gene of *Pseudomonas stutzeri* is introduced into *E. coli*, maltose is converted into glucose by the trehalase present in *E. coli*. As the resulting glucose is metabolized, pH decreases and thereby the color of the colonies on the MacConkey agar medium changes from yellowish to red. This principle was applied to the present cloning system. The transformed *E. coli* ATCC35467 with the genomic library was cultured on a MacConkey agar medium to obtain red colonies. The isolation of plasmid DNA revealed that it contained about 4.7 kb DNA fragment. The plasmid was designated as pCJ104. To assay enzymes, *E. coli* ATCC35467/pUC18 (control), *E. coli* ATCC35467/pCJ104 and wild type *Pseudomonas stutzeri* CJ38 were cultured. *E. coli* cells were grown on a LB medium until their early resting stage. *Pseudomonas stutzeri* CJ38 was grown on a nutrient medium. The cells were separated by centrifugation and crushed. The crushed cells were reacted with 20% maltose as substrate in 20 mM diethanolamine as buffer

solution, at pH of 8.5 to 9.0 and a temperature of 35°C. 1.0% trichloroacetic acid was added to the reaction solution, which was then subjected to centrifugation and high performance liquid chromatography to assay the quantities of maltose and trehalose. The results are shown in Table 3 below.

5 Table 3. Enzyme Titration

Microorganisms	Non-enzymatic activities (U*/mg of protein)	Culture Titer (U/ml of culture solution)
<i>Pseudomonas stutzeri</i> CJ38	0.1	0.023
<i>E. coli</i> ATCC35467/pUC18	0	0
<i>E. coli</i> ATCC35467/pCJ104	0.26	0.175

\*U=μmol trehalose/minutes

### 15 Example 7

#### Restriction Map Construction of Trehalose Synthase Gene (Figure 5)

20 The plasmid pCJ104 was separated using conventional methods and treated with various restriction enzymes to construct a restriction map.

The plasmid pCJ104 was subjected to single, double, and triple-digest procedures using about twenty restriction enzymes, such as *AatII*, *BamHI*, *EcoRI*, *EcoRV*, *KpnI*, *NcoI*, *NdeI*, *PstI*, *SacI*, *SacII*, *SalI*, *SphI* and *XhoI*. DNA fragments were  
25 analyzed by electrophoresis through agarose gel and compared to construct the restriction map.

### Example 3

#### Subcloning of Trehalose Synthase Gene and Enzyme Assay

30

(1) Subcloning of Trehalose Synthase Gene (Figure 6)

A subcloning was carried out to determine the sites of the trehalose synthase gene in 4.7 kb plasmid pCJ104. The plasmid pCJ104 was cleaved with *KpnI* and a  
5 3.35 kb fragment was isolated. This fragment was introduced into vector pUC18/*KpnI*/CIP and *E. coli* NM522 was transformed with the resulting recombinant. The recombinant plasmid pCJ121 with a directional cloning of 3.35 kb fragment into pUC18/*KpnI* was constructed. In addition, the plasmid pCJ104 was cleaved with double digestions of *Bam*HI and *Bgl*II. The 2.5 kb *Bam*HI-*Bgl*II fragment thus  
10 obtained was purified and ligated into pUC18/*Bam*HI/CIP, followed by transformation of *E. coli* NM522 with the recombinant. The recombinant plasmid pCJ122 with directional cloning of 2.5 kb *Bam*HI-*Bgl*II fragment into pUC18/*Bam*HI was constructed. Finally, the plasmid pCJ104 was double-digested with *Bam*HI and *Eco*RI and the resulting 1.2 kb *Bam*HI-*Eco*RI fragment was purified. This fragment was  
15 ligated into vector pUC18/*Bam*HI/*Eco*RI and *E. coli* NM522 was transformed with the recombinant. The recombinant plasmid pCJ123 was constructed.

*E. coli* ATCC35467 was transformed with each of the constructed recombinant plasmids. The transformants were cultured on a MacConkey-ampicilline agar medium  
20 containing 2.0% maltose (20 g/L) and the color of the colonies formed therefrom was observed. It was observed that the *E. coli* ATCC35467 carrying pCJ121 and pCJ122 formed red colonies but that the *E. coli* ATCC35467 carrying pCJ123 formed yellow colonies since it did not decompose maltose. Therefore, it can be seen that the trehalose synthase gene is located in the larger 2.5 kb *Bam*HI-*Bgl*II fragment, rather  
25 than in the 1.2 kb *Bam*HI-*Eco*RI fragment.

(2) Titration of Trehalose Synthase of Transformant Containing Subcloned Plasmid

Transformed *E. coli* ATCC35467/pCJ121, ATCC35467/pCJ122 and  
30 ATCC35467/pCJ123 were cultured on an LB-Ap medium until the early resting stage. The cells were recovered by centrifugation and washed twice with an appropriate volume of 20 mM diethanolamine solution. The washed cells were suspended in an

appropriate volume of 20 mM diethanolamine solution and crushed by ultrasonicator. The crushed cells were centrifuged and the supernatant obtained therefrom was used as enzymatic liquid. The supernatant was reacted with 20% maltose solution containing 20 mM diethanolamine, pH 8.5 to 9.0 at 35°C. 1.0% trichloroacetic acid was added to the reaction solution, and centrifugation and HPLC were conducted for analysis. One unit of enzyme activity was defined as a quantity of enzyme when it produced 1  $\mu$ mol of trehalose per minute. The results are shown in Table 5 below.

According to the double titration, the enzyme titer of *E. coli* ATCC35467/pCJ122 was the highest. *E. coli* ATCC35467/pCJ122 was cultured in high density under the conditions described in Table 6 below in 5 L fermenter. As a result, the non-enzymatic activity was 5.0 U/mg of protein, equal to that obtained by culturing it on an LB medium, and the titer of the trehalose synthase enzyme in the high density culture was increased to 30 U/ml of culture (Table 5). The non-enzymatic activity and culture titer of *E. coli* ATCC35467/pCJ122 were increased 50 times and about 1,300 times, respectively, compared to wild type *Pseudomonas stutzeri*.

Table 5

Microorganisms	Non-enzymatic Activity (U/mg of protein)	Culture Titer of 5 L Fermenter (U/ml of culture)
<i>E. coli</i> ATCC35467/pCJ121	0.43	-
<i>E. coli</i> ATCC35467/pCJ122	4.95	30
<i>E. coli</i> ATCC35467/pCJ123	0	-

Table 6

Fermentation Medium	g/L	Culture Condition
glycerol	50	pH 7.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6	Temperature of 33°C
KH <sub>2</sub> PO <sub>4</sub>	2	800 rpm

MgSO <sub>4</sub> ·7H <sub>2</sub> O	1	1.0 vvm
Yeast Extract	5	
Trace Elements	1 ml	
Amino Acids (Threonine, Leucine, Isoleucine, Valine, Histidine, Arginine)	0.5	

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3 <110> APPLICANT: Lee, Se Yong et al.

5 <120> TITLE OF INVENTION: Trehalose Synthase Protein, Gene, Plasmids, Microorganisms,  
and A Process

6 for Producing Trehalose

8 <130> FILE REFERENCE: 1599-0206P

10 <140> CURRENT APPLICATION NUMBER: US 09/937,215

11 <141> CURRENT FILING DATE: 2001-09-24

13 <160> NUMBER OF SEQ ID NOS: 6

15 <170> SOFTWARE: PatentIn version 3.1

17 <210> SEQ ID NO: 1

18 <211> LENGTH: 4753

19 <212> TYPE: DNA

20 <213> ORGANISM: Pseudomonas stutzeri

22 <220> FEATURE:

23 <221> NAME/KEY: CDS

24 <222> LOCATION: (1537)..(3603)

25 <223> OTHER INFORMATION:

28 <400> SEQUENCE: 1

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DATE: 03/05/2002

PATENT APPLICATION: US/09/937,215

TIME: 13:58:25

Input Set : A:\1599-0206P.ST25.txt

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VERIFICATION SUMMARY

DATE: 03/05/2002

PATENT APPLICATION: US/09/937,215

TIME: 13:58:26

Input Set : A:\1599-0206P.ST25.txt

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TOTAL 24650

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<120> Trehalose Synthase Protein, Gene, Plasmids, Microorganisms, and A Process for Producing Trehalose

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WO 00/56868

PCT/KR99/00131

1.

SEQUENCE LISTING

GENERAL INFORMATION:

APPLICANT

NAME: Cheil Jedang Corporation  
STREET:  
CITY: Seoul  
COUNTRY: Republic of Korea  
POSTAL CODE (ZIP): 100-095  
TELEPHONE: 82 2 7268 286  
TELEFAX: 82 2 7268 219  
TELEX:

TITLE OF INVENTION: Trehalose Synthase Protein, Gene, Plasmids,  
Microorganisms, and A Process for Producing Trehalose

NUMBER OF SEQUENCES: 1

CORRESPONDENCE ADDRESS:

ADDRESS: 500, 5-ga, Namdaemun-ro, Chung-ku  
STREET:  
CITY: Seoul  
STATE OR PROVINCE:  
COUNTRY: Republic of Korea  
POSTAL CODE: 100-095

COMPUTER READABLE FORM:

MEDIUM TYPE : Floppy disk  
COMPUTER: IBM PC Compatible  
OPERATING SYSTEM: Windows 95  
SOFTWARE: Notepad, Hangul 97

CURRENT APPLICATION DATA:

APPLICATION NUMBER:  
FILING DATE:  
CLASSIFICATION:

PRIOR APPLICATION DATA:

COUNTRY: Republic of Korea  
APPLICATION NUMBER:

FILING DATE:  
CLASSIFICATION:

## ATTORNEY/AGENT INFORMATION:

NAME: Choi, Hak Hyun and Hwang, Ju Myung  
REGISTRATION NUMBER:  
REFERENCE/DOCKET NUMBER:

## TELECOMMUNICATION INFORMATION:

TELEPHONE: 82 2 365 2727  
TELEFAX: 82 2 365 3370  
ELECTRONIC MAIL: patent@hmpj.com

## INFORMATION FOR SEQ ID NO: 1

## SEQUENCE CHARACTERISTICS:

LENGTH: 4753  
TYPE: nucleic acid  
STRANDEDNESS: double  
TOPOLOGY: linear

MOLECULE TYPE: Trehalose Synthase Gene

HYPOTHETICAL:

ANTI-SENSE:

## ORIGINAL SOURCE:

ORGANISM: *Pseudomonas stutzeri*  
STRAIN: CJ38

## SEQUENCE DESCRIPTION: SEQ ID NO: 1

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ACACGCCGAG GCCATAGCCC ACCGCCAGCG CCGCGCCGGC CAGCACGCCC TGGCTCAGCG	1260
TCGAGCGCGG CAGCAGCGAT GGGTCAGCG CCGCGCAGAA GAACAGCGTG CCCAGCAGCA	1320
GGCCGAAACC GGACAGCGAG CGCCAGATAT AGAGGACGGG CAGGTGCAGC ATGAAGATCT	1380
CCGCGGTCGG GTGACGGCGT CGCGCCTCGG CATATCGAGG CGTGTCGGT CGTGCGGTTT	1440
CCGTGATGGT CCGCAGCAGG CCAATCCGAT GCAACGATGG CCGAGCGGCC GACTCAAACG	1500
TCTACATTTC CTAGTGCTG CCGGAACCGA TCGCCG	1536
ATG AGC ATC CCA GAC AAC ACC TAT ATC GAA TGG CTG GTC AGC CAG TCC	1584
Met Ser Ile Pro Asp Asn Thr Tyr Ile Glu Trp Leu Val Ser Gln Ser	
ATG CTG CAT GCG GCC CGC GAG CGG TCG CGT CAT TAC GCC GGC CAG GCG	1632
Met Leu His Ala Ala Arg Glu Arg Ser Arg His Tyr Ala Gly Gln Ala	
CGT CTC TGG CAG CGG CCT TAT GCC CAG GCC CGC CGC GAT GCC AGC	1680
Arg Leu Trp Gln Arg Pro Try Ala Gln Ala Arg Pro Arg Asp Ala Ser	
GCC ATC GCC TCG GTG TGG TTC ACC GCC TAT CCG GCG GCC ATC ATC ACG	1728
Ala Ile Ala Ser Val Trp Phe Thr Ala Tyr Pro Ala Ala Ile Ile Thr	
CCG GAA GGC GGC ACG GTA CTC GAG GCC CTC GGC GAC GAC CGC CTC TGG	1776
Pro Glu Gly Gly Thr Val Leu Glu Ala Leu Gly Asp Asp Arg Leu Trp	
AGT GCG CTC TCC GAA CTC GGC GTG CAG GGC ATC CAC AAC GGG CCG ATG	1824
Ser Ala Leu Ser Glu Leu Gly Val Gln Gly Ile His Asn Gly Pro Met	
AAG CGT TCC GGT GGC CTG CGC GGA CGC GAG TTC ACC CCG ACC ATC GAC	1872
Lys Arg Ser Gly Gly Leu Arg Gly Arg Glu Phe Thr Pro Thr Ile Asp	
GGC AAC TTC GAC CGC ATC AGC TTC GAT ATC GAC CCG AGC CTG GGG ACC	1920
Gly Asn Phe Asp Arg Ile Ser Phe Asp Ile Asp Pro Ser Leu Gly Thr	
GAG GAG CAG ATG CTG CAG CTC AGC CGG GTG GCC GCG GCG CAC AAC GCC	1968
Glu Glu Gln Met Leu Gln Leu Ser Arg Val Ala Ala Ala His Asn Ala	

4.

ATC GTC ATC GAC GAC ATC GTG CCG GCA CAC ACC GGC AAG GGT GCC GAC Ile Val Ile Asp Asp Ile Val Pro Ala His Thr Gly Lys Gly Ala Asp	2016
TTC CGC CTC GCG GAA ATG GCC TAT GGC GAC TAC CCC GGG CTG TAC CAC Phe Arg Leu Ala Glu Met Ala Tyr Gly Asp Tyr Pro Gly Leu Tyr His	2064
ATG GTG GAA ATC CGC GAG GAG GAC TGG GAG CTG CTG CCC GAG GTG CCG Met Val Glu Ile Arg Glu Glu Asp Trp Glu Leu Leu Pro Glu Val Pro	2112
GCC GGG CGT GAT TCG GTC AAC CTG CTG CCG CCG GTG GTC GAC CGG CTC Ala Gly Arg Asp Ser Val Asn Leu Leu Pro Pro Val Val Asp Arg Leu	2160
AAG GAA AAG CAC TAC ATC GTC GGC CAG CTG CAG CGG GTG ATC TTC TTC Lys Glu Lys His Tyr Ile Val Gly Gln Leu Gln Arg Val Ile Phe Phe	2208
GAG CCG GGC ATC AAG GAC ACC GAC TGG AGC GTC ACC GGC GAG GTC ACC Glu Pro Gly Ile Lys Asp Thr Asp Trp Ser Val Thr Gly Glu Val Thr	2256
GGG GTC GAC GGC AAG GTG CGT CGC TGG GTC TAT CTG CAC TAC TTC AAG Gly Val Asp Gly Lys Val Arg Arg Trp Val Tyr Leu His Tyr Phe Lys	2304
GAG GGC CAG CCG TCG CTG AAC TGG CTC GAC CCG ACC TTC GCC GCG CAG Glu Gly Gln Pro Ser Leu Asn Trp Leu Asp Pro Thr Phe Ala Ala Gln	2352
CAG CTG ATC ATC GGC GAT GCG CTG CAC GCC ATC GAC GTC ACC GGC GCC Gln Leu Ile Ile Gly Asp Ala Leu His Ala Ile Asp Val Thr Gly Ala	2400
CGG GTG CTG CGC CTG GAC GCC AAC GGC TTC CTC GGC GTG GAA CGG CGC Arg Val Leu Arg Leu Asp Ala Asn Gly Phe Leu Gly Val Glu Arg Arg	2448
GCC GAG GGC ACG GCC TGG TCG GAG GGC CAC CCG CTG TCC GTC ACC GGC Ala Glu Gly Thr Ala Trp Ser Glu Gly His Pro Leu Ser Val Thr Gly	2496
AAC CAG CTG CTC GCC GGG GCG ATC CGC AAG GCC GGC GGC TTC AGC TTC Asn Gln Leu Leu Ala Gly Ala Ile Arg Lys Ala Gly Gly Phe Ser Phe	2544
CAG GAG CTG AAC CTG ACC ATC GAT GAC ATC GCC GCC ATG TCC CAC GGC Gln Glu Leu Asn Leu Thr Ile Asp Asp Ile Ala Ala Met Ser His Gly	2592
GGG GCC GAT CTG TCC TAC GAC TTC ATC ACC CGC CCG GCC TAT CAC CAT Gly Ala Asp Leu Ser Tyr Asp Phe Ile Thr Arg Pro Ala Tyr His His	2640
GCG TTG CTC ACC GGC GAT ACC GAA TTC CTG CGC ATG ATG CTG CGC GAA Ala Leu Leu Thr Gly Asp Thr Glu Phe Leu Arg Met Met Leu Arg Glu	2688

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5:

GTG CAC GCC TTC GGC ATC GAC CCG GCG TCA CTG ATC CAT GCG CTG CAG Val His Ala Phe Gly Ile Asp Pro Ala Ser Leu Ile His Ala Leu Gln	2736
AAC CAT GAC GAG TTG ACC CTG GAG CTG GTG CAC TTC TGG ACG CTG CAC Asn His Asp Glu Leu Thr Leu Glu Leu Val His Phe Trp Thr Leu His	2784
GCC TAC GAC CAT TAC CAC TAC AAG GGC CAG ACC CTG CCC GGC GGC CAC Ala Tyr Asp His Tyr His Tyr Lys Gly Gln Thr Leu Pro Gly Gly His	2832
CTG CGC GAA CAT ATC CGC GAG GAA ATG TAC GAG CGG CTG ACC GGC GAA Leu Arg Glu His Ile Arg Glu Glu Met Tyr Glu Arg Leu Thr Gly Glu	2880
CAC GCG CCG TAC AAC CTC AAG TTC GTC ACC AAC GGG GTG TCC TGC ACC His Ala Pro Tyr Asn Leu Lys Phe Val Thr Asn Gly Val Ser Cys Thr	2928
ACC GCC AGC GTG ATC GCC GCG GCG CTT AAC ATC CGT GAT CTG GAC GCC Thr Ala Ser Val Ile Ala Ala Ala Leu Asn Ile Arg Asp Leu Asp Ala	2976
ATC GGC CCG GCC GAG GTG GAG CAG ATC CAG CGT CTG CAT ATC CTG CTG Ile Gly Pro Ala Glu Val Glu Gln Ile Gln Arg Leu His Ile Leu Leu	3024
GTG ATG TTC AAT GCC ATG CAG CCC GGC GTG TTC GCC CTC TCC GGC TGG Val Met Phe Asn Ala Met Gln Pro Gly Val Phe Ala Leu Ser Gly Trp	3072
GAT CTG GTC GGC GCC CTG CCG CTG GCG CCC GAG CAG GTC GAG CAC CTG Asp Leu Val Gly Ala Leu Pro Leu Ala Pro Glu Gln Val Glu His Leu	3120
ATG GGC GAT GGC GAT ACC CGC TGG ATC AAT CGC GGC GGC TAT GAC CTC Met Gly Asp Gly Asp Thr Arg Trp Ile Asn Arg Gly Gly Tyr Asp Leu	3168
GCC GAT CTG GCG CCG GAG GCG TCG GTC TCC GCC GAA GGC CTG CCC AAG Ala Asp Leu Ala Pro Glu Ala Ser Val Ser Ala Glu Gly Leu Pro Lys	3216
GCC CGC TCG CTG TAC GGC AGC CTG GCC GAG CAG CTG CAG CGG CCA GGC Ala Arg Ser Leu Tyr Gly Ser Leu Ala Glu Gln Leu Gln Arg Pro Gly	3264
TCC TTC GCC TGC CAG CTC AAG CGC ATC CTC AGC GTG CGC CAG GCC TAC Ser Phe Ala Cys Gln Leu Lys Arg Ile Leu Ser Val Arg Gln Ala Tyr	3312
GAC ATC GCT GCC AGC AAG CAG ATC CTG ATT CCG GAT GTG CAG GCG CCG Asp Ile Ala Ala Ser Lys Gln Ile Leu Ile Pro Asp Val Gln Ala Pro	3360
GGA CTC CTG GTG ATG GTC CAC GAG CTG CCT GCC GGC AAG GGC GTG CAG Gly Leu Leu Val Met Val His Glu Leu Pro Ala Gly Lys Gly Val Gln	3408

6/

CTC ACG GCA CTG AAC TTC AGC GCC GAG CCG GTC AGC GAG ACC ATC TGC Leu Thr Ala Leu Asn Phe Ser Ala Glu Pro Val Ser Glu Thr Ile Cys	3456
CTG CCC GGC GTG GCG CCC GGC CCG GTG GTG GAC ATC ATT CAC GAG AGT Leu Pro Gly Val Ala Pro Gly Pro Val Val Asp Ile Ile His Glu Ser	3504
GTG GAG GGC GAC CTC ACC GAC AAC TGC GAG CTG CAG ATC AAC CTC GAC Val Glu Gly Asp Leu Thr Asp Asn Cys Glu Leu Gln Ile Asn Leu Asp	3552
CCG TAC GAG GGG CTT GCC CTG CGT GTG GTG AGC GCC GCG CCG CCG GTG Pro Tyr Glu Gly Leu Ala Leu Arg Val Val Ser Ala Ala Pro Pro Val	3600
ATC TGA GCCC Ile	3610
CCTCTTCGCG CGCCCGGGT CCGCCGCTAT AGTGGCGAGC GCCTGGGGCG CGCATTGCCC	3670
TCGCCGTGCA GACCAGCCCG TGTCGTTTAC TTCGCTTTTC CGCCTTGCGC TGCTGCCGCT	3730
GGCGCTGCTT GCCGACCCG TCTGGGCGCA GACCGCTGC CCGCCCGGCC AGCAGCCGAT	3790
CTGCCTGAGC GGCAGCTGCC TCTGCGTGCC GGCCGCCGCC AGCGATCCAC AGGCGGTCTA	3850
CGACCGCGTG CAGCGTATGG CTACGCTGGC CCTGCAGAAC TGGATCCAGC AGTCGCGCGA	3910
CCGCCTGATG GCCGGCGGGC TCGAGCCGAT ACCGCTGCAC ATCCGCTCGC AGCTCGAGCC	3970
GTATTTGAT CTTGCCGTGC TGGAGAGTGC GCGGTACCGC GTCGGCGACG AGGTGGTGCT	4030
GACTGCCGGC AACACCCCTGC TCGCAACCC GGACGTCAAT GCCGTGACCC TGATCGACGT	4090
CATGCTCTTC CGCCACGAGG AGGATGCCCG GGACAACTC GCGCTCTGGG CCCATGAGCT	4150
CAAGCACGTC GAGCAATATC TGGACTGGGG CGTCGCCGAG TTCGCCCGGC GCTATACGCA	4210
GGATTTCCGT GCCGTGGAGC GCCCGGCCCTA TGGCTGGAG CGTGAGGTGG AAGAGGCCCT	4270
GCGCGAGACG CAGACGCGGC GCTGAGCGAG CTGATCGGTG CTGCTGCCCG CACTGGGCTG	4330
AAGCCACCA ATGACGCCGG CGAAACGAA AAACCCCGCC GAGGCGGGT TTCTGACGCG	4390
GGTTGTGCGG TCAGCTCAGA ACGCCGGGAC CACGGCGCCC TTGTACTTTT CCTCGATGAA	4450
CTGGCGTACT TGCTCGCTGT GCAGCGCGGC AGCCAGTTTC TGCATGGCAT CGCTGTCCTT	4510
GTGTCCGGA CGGGCGACCA GAATGTTTAC GTATGGCGAG TCGTGCCCT CGATCACCAG	4570
GGCGTCTGG GTGGGTTCA GCTTGGCTTC CAGCGGTAG TTGGTGTGA TCAGCGCCAG	4630
GTCGACCTGG GTCAGCACGC GCGGCAGAGT CGCGGCTTCC AGTTCGCGGA TCTTGATCTT	4690
CTTCGGGTTT TCGGCGATGT CTTCGGCGTG GCGGTGATGC CGGCGCCGTC CTTGAGCCG	4750
ATC	4753

T04260" 5124650

SEQUENCE LISTING

GENERAL INFORMATION:

APPLICANT

NAME: Cheil Jedang Corporation  
 STREET:  
 CITY: Seoul  
 COUNTRY: Republic of Korea  
 POSTAL CODE (ZIP): 100-095  
 TELEPHONE: 82 2 7268 286  
 TELEFAX: 82 2 7268 219  
 TELEX:

TITLE OF INVENTION: Trehalose Synthase Protein, Gene, Plasmids,  
 Microorganisms, and A Process for Producing Trehalose

NUMBER OF SEQUENCES: 1

CORRESPONDENCE ADDRESS:

ADDRESS: 500, 5-ga, Namdaemun-ro, Chung-ku  
 STREET:  
 CITY: Seoul  
 STATE OR PROVINCE:  
 COUNTRY: Republic of Korea  
 POSTAL CODE: 100-095

COMPUTER READABLE FORM:

MEDIUM TYPE : Floppy disk  
 COMPUTER: IBM PC Compatible  
 OPERATING SYSTEM: Windows 95  
 SOFTWARE: Notepad, Hangul 97

CURRENT APPLICATION DATA:

APPLICATION NUMBER:  
 FILING DATE:  
 CLASSIFICATION:

PRIOR APPLICATION DATA:

COUNTRY: Republic of Korea  
 APPLICATION NUMBER:

704260-5722650

FILING DATE:  
CLASSIFICATION:

## ATTORNEY/AGENT INFORMATION:

NAME: Choi, Hak Hyun and Hwang, Ju Myung  
REGISTRATION NUMBER:  
REFERENCE/DOCKET NUMBER:

## TELECOMMUNICATION INFORMATION:

TELEPHONE: 82 2 365 2727  
TELEFAX: 82 2 365 3370  
ELECTRONIC MAIL: patent@hmpj.com

## INFORMATION FOR SEQ ID NO: 1

## SEQUENCE CHARACTERISTICS:

LENGTH: 4753  
TYPE: nucleic acid  
STRANDEDNESS: double  
TOPOLOGY: linear

MOLECULE TYPE: Trehalose Synthase Gene

HYPOTHETICAL:

ANTI-SENSE:

## ORIGINAL SOURCE:

ORGANISM: *Pseudomonas stutzeri*  
STRAIN: CJ38

## SEQUENCE DESCRIPTION: SEQ ID NO: 1

GATCGCTGGC	GTACTGCAGG	TAGAGCAGGC	GCATCGGCCC	CCAGGGCGCA	TCGGCCGGCT	60
CCGCTGTGCC	CTGCTGGTTC	ATGAAGCGGA	CGAAGCGGCC	ATCGCGGAAC	CGTGGACGCC	120
ATTCGGGGCT	GTCCGGGTCG	CGGCTGTCGG	TGAGCGTGCG	CCACAGGTCG	CTGCGAAACG	180
GCGGACCGCT	CCAAAGCGCG	CCGTGGATGG	GATCGCCGAG	CAGTTCGTGC	AGCTCCCAGG	240
AACGTTGCCA	ATGCAGCGCG	CCGAGGCTCA	GGCCATGCAG	ATACAGGCGC	GGTCGGCGTT	300
CGGCCGGCAG	TTCGGTCCAG	TAGCCATAGA	TCTCGGCGAA	TAGCGCGCGG	GCCACGTCGC	360
GGCCGTAGTC	GGCCTCCACC	AGCAGCGCCA	GCGGGCTGTT	CAGATAGGAG	TACTGCAACG	420
CCACGCTGGC	GATATCGCCG	TGGTGCAGGT	ATTCCACTGC	GTTTCATCGCC	GCCGGGTCGA	480
TCCAGCCGGT	ACCGGTGGGC	GTCACCAGCA	CCAGCACCAG	TCGCTCGAAG	GCGCCGCTGC	540

GCTGCAGCTC GCGCAAGGCC AGACGCGCCC GCTGGCGCGG GGTCTCTGCC GCGCGCAGAC	600
CGACGTAGAC GCGAATCGGC TCGAGCGCCG AGCGGCCGCT CAAGACGCTG ATATCCGCCG	660
CCGACGGGCC GGAGCCGATG AACTCGCGGC CGGTGCGGCC CAGCTCCTCC CAGCGCAGCA	720
ACGAGGCCCG GCTGCCGCTT TTCAGCGGCG AGGCCGGTGG CGCCGTCTCC GGTTCGATCA	780
GGGCGTCGTA CTGCGCGAAG GATGCGTCCA GCATGCGCAG TGCCCGCGCC GCCAGCACAT	840
CGCTGAGCAG CGACCAGAAC AGCGCCAGCG CCACCAGCAC GCCGATCAGG TTGGCCAGGC	900
GCCGTGGCAG CACGCGGTGCG GCGTGCCGCG AGACGAAGCG CGACACCAGC CGATACAGAC	960
GCGCCAGCGT CAGCAGGATG AGAAAGGTCG CCAGCGCGGT GAGAATGACT TCGAGCAGGT	1020
GCGCACTGCT CACCGGCGGC ATGCCCATCA GCGCGCGTAC CGCGTTCTGC CAGCCGGCGA	1080
CCTGGCTGAG GAAATACCCG GCCAGCAGCA GGCAGCCGAC CGCGATCAGC AGATTGACCC	1140
GCTCGCGCTG CCAGCCTGGG CGTCCGGCA GTTCCAGATA GCGCCACAGC CAGCGCCAGA	1200
ACACGCCGAG GCCATAGCCC ACCGCCAGCG CCGCGCCGGC CAGCACGCC TGGCTCAGCG	1260
TCGAGCGCGG CAGCAGCGAT GCGTCAGCG CCGCGCAGAA GAACAGCGTG CCCAGCAGCA	1320
GGCCGAAACC GGACAGCGAG CGCCAGATAT AGAGGACGGG CAGGTGCAGC ATGAAGATCT	1380
CCGCGGTGCG GTGACGGCGT CGCGCCTCGG CATATCGAGG CGTGTCCGGT CGTGCGGTTC	1440
CCGTGATGGT CCGCAGCAGG CCAATCCGAT GCAACGATGG CCGAGCGGCC GACTCAAACG	1500
TCTACATTTC CCTAGTGCTG CCGGAACCGA TCGCCG	1536
ATG AGC ATC CCA GAC AAC ACC TAT ATC GAA TGG CTG GTC AGC CAG TCC	1584
Met Ser Ile Pro Asp Asn Thr Tyr Ile Glu Trp Leu Val Ser Gln Ser	
ATG CTG CAT GCG GCC CGC GAG CGG TCG CGT CAT TAC GCC GGC CAG GCG	1632
Met Leu His Ala Ala Arg Glu Arg Ser Arg His Tyr Ala Gly Gln Ala	
CGT CTC TGG CAG CGG CCT TAT GCC CAG GCC CGC CCG CGC GAT GCC AGC	1680
Arg Leu Trp Gln Arg Pro Try Ala Gln Ala Arg Pro Arg Asp Ala Ser	
GCC ATC GCC TCG GTG TGG TTC ACC GCC TAT CCG GCG GCC ATC ATC ACG	1728
Ala Ile Ala Ser Val Trp Phe Thr Ala Tyr Pro Ala Ala Ile Ile Thr	
CCG GAA GGC GGC ACG GTA CTC GAG GCC CTC GGC GAC GAC CGC CTC TGG	1776
Pro Glu Gly Gly Thr Val Leu Glu Ala Leu Gly Asp Asp Arg Leu Trp	
AGT GCG CTC TCC GAA CTC GGC GTG CAG GGC ATC CAC AAC GGG CCG ATG	1824
Ser Ala Leu Ser Glu Leu Gly Val Gln Gly Ile His Asn Gly Pro Met	
AAG CGT TCC GGT GGC CTG CGC GGA CGC GAG TTC ACC CCG ACC ATC GAC	1872
Lys Arg Ser Gly Gly Leu Arg Gly Arg Glu Phe Thr Pro Thr Ile Asp	
GGC AAC TTC GAC CGC ATC AGC TTC GAT ATC GAC CCG AGC CTG GGG ACC	1920
Gly Asn Phe Asp Arg Ile Ser Phe Asp Ile Asp Pro Ser Leu Gly Thr	
GAG GAG CAG ATG CTG CAG CTC AGC CGG GTG GCC GCG GCG CAC AAC GCC	1968
Glu Glu Gln Met Leu Gln Leu Ser Arg Val Ala Ala Ala His Asn Ala	

4.

ATC GTC ATC GAC GAC ATC GTG CCG GCA CAC ACC GGC AAG GGT GCC GAC Ile Val Ile Asp Asp Ile Val Pro Ala His Thr Gly Lys Gly Ala Asp	2016
TTC CGC CTC GCG GAA ATG GCC TAT GGC GAC TAC CCC GGG CTG TAC CAC Phe Arg Leu Ala Glu Met Ala Tyr Gly Asp Tyr Pro Gly Leu Tyr His	2064
ATG GTG GAA ATC CGC GAG GAG GAC TGG GAG CTG CTG CCC GAG GTG CCG Met Val Glu Ile Arg Glu Glu Asp Trp Glu Leu Leu Pro Glu Val Pro	2112
GCC GGG CGT GAT TCG GTC AAC CTG CTG CCG CCG GTG GTC GAC CGG CTC Ala Gly Arg Asp Ser Val Asn Leu Leu Pro Pro Val Val Asp Arg Leu	2160
AAG GAA AAG CAC TAC ATC GTC GGC CAG CTG CAG CGG GTG ATC TTC TTC Lys Glu Lys His Tyr Ile Val Gly Gln Leu Gln Arg Val Ile Phe Phe	2208
GAG CCG GGC ATC AAG GAC ACC GAC TGG AGC GTC ACC GGC GAG GTC ACC Glu Pro Gly Ile Lys Asp Thr Asp Trp Ser Val Thr Gly Glu Val Thr	2256
GGG GTC GAC GGC AAG GTG CGT CGC TGG GTC TAT CTG CAC TAC TTC AAG Gly Val Asp Gly Lys Val Arg Arg Trp Val Tyr Leu His Tyr Phe Lys	2304
GAG GGC CAG CCG TCG CTG AAC TGG CTC GAC CCG ACC TTC GCC GCG CAG Glu Gly Gln Pro Ser Leu Asn Trp Leu Asp Pro Thr Phe Ala Ala Gln	2352
CAG CTG ATC ATC GGC GAT GCG CTG CAC GCC ATC GAC GTC ACC GGC GCC Gln Leu Ile Ile Gly Asp Ala Leu His Ala Ile Asp Val Thr Gly Ala	2400
CGG GTG CTG CGC CTG GAC GCC AAC GGC TTC CTC GGC GTG GAA CGG CGC Arg Val Leu Arg Leu Asp Ala Asn Gly Phe Leu Gly Val Glu Arg Arg	2448
GCC GAG GGC ACG GCC TGG TCG GAG GGC CAC CCG CTG TCC GTC ACC GGC Ala Glu Gly Thr Ala Trp Ser Glu Gly His Pro Leu Ser Val Thr Gly	2496
AAC CAG CTG CTC GCC GGG GCG ATC CGC AAG GCC GGC GGC TTC AGC TTC Asn Gln Leu Leu Ala Gly Ala Ile Arg Lys Ala Gly Gly Phe Ser Phe	2544
CAG GAG CTG AAC CTG ACC ATC GAT GAC ATC GCC GCC ATG TCC CAC GGC Gln Glu Leu Asn Leu Thr Ile Asp Asp Ile Ala Ala Met Ser His Gly	2592
GGG GCC GAT CTG TCC TAC GAC TTC ATC ACC CGC CCG GCC TAT CAC CAT Gly Ala Asp Leu Ser Tyr Asp Phe Ile Thr Arg Pro Ala Tyr His His	2640
GCG TTG CTC ACC GGC GAT ACC GAA TTC CTG CGC ATG ATG CTG CGC GAA Ala Leu Leu Thr Gly Asp Thr Glu Phe Leu Arg Met Met Leu Arg Glu	2688

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5:

GTG CAC GCC TTC GGC ATC GAC CCG GCG TCA CTG ATC CAT GCG CTG CAG Val His Ala Phe Gly Ile Asp Pro Ala Ser Leu Ile His Ala Leu Gln	2736
AAC CAT GAC GAG TTG ACC CTG GAG CTG GTG CAC TTC TGG ACG CTG CAC Asn His Asp Glu Leu Thr Leu Glu Leu Val His Phe Trp Thr Leu His	2784
GCC TAC GAC CAT TAC CAC TAC AAG GGC CAG ACC CTG CCC GGC GGC CAC Ala Tyr Asp His Tyr His Tyr Lys Gly Gln Thr Leu Pro Gly Gly His	2832
CTG CGC GAA CAT ATC CGC GAG GAA ATG TAC GAG CGG CTG ACC GGC GAA Leu Arg Glu His Ile Arg Glu Glu Met Tyr Glu Arg Leu Thr Gly Glu	2880
CAC GCG CCG TAC AAC CTC AAG TTC GTC ACC AAC GGG GTG TCC TGC ACC His Ala Pro Tyr Asn Leu Lys Phe Val Thr Asn Gly Val Ser Cys Thr	2928
ACC GCC AGC GTG ATC GCC GCG GCG CTT AAC ATC CGT GAT CTG GAC GCC Thr Ala Ser Val Ile Ala Ala Ala Leu Asn Ile Arg Asp Leu Asp Ala	2976
ATC GGC CCG GCC GAG GTG GAG CAG ATC CAG CGT CTG CAT ATC CTG CTG Ile Gly Pro Ala Glu Val Glu Gln Ile Gln Arg Leu His Ile Leu Leu	3024
GTG ATG TTC AAT GCC ATG CAG CCC GGC GTG TTC GCC CTC TCC GGC TGG Val Met Phe Asn Ala Met Gln Pro Gly Val Phe Ala Leu Ser Gly Trp	3072
GAT CTG GTC GGC GCC CTG CCG CTG GCG CCC GAG CAG GTC GAG CAC CTG Asp Leu Val Gly Ala Leu Pro Leu Ala Pro Glu Gln Val Glu His Leu	3120
ATG GGC GAT GGC GAT ACC CGC TGG ATC AAT CGC GGC GGC TAT GAC CTC Met Gly Asp Gly Asp Thr Arg Trp Ile Asn Arg Gly Gly Tyr Asp Leu	3168
GCC GAT CTG GCG CCG GAG GCG TCG GTC TCC GCC GAA GGC CTG CCC AAG Ala Asp Leu Ala Pro Glu Ala Ser Val Ser Ala Glu Gly Leu Pro Lys	3216
GCC CGC TCG CTG TAC GGC AGC CTG GCC GAG CAG CTG CAG CGG CCA GGC Ala Arg Ser Leu Tyr Gly Ser Leu Ala Glu Gln Leu Gln Arg Pro Gly	3264
TCC TTC GCC TGC CAG CTC AAG CGC ATC CTC AGC GTG CGC CAG GCC TAC Ser Phe Ala Cys Gln Leu Lys Arg Ile Leu Ser Val Arg Gln Ala Tyr	3312
GAC ATC GCT GCC AGC AAG CAG ATC CTG ATT CCG GAT GTG CAG GCG CCG Asp Ile Ala Ala Ser Lys Gln Ile Leu Ile Pro Asp Val Gln Ala Pro	3360
GGA CTC CTG GTG ATG GTC CAC GAG CTG CCT GCC GGC AAG GGC GTG CAG Gly Leu Leu Val Met Val His Glu Leu Pro Ala Gly Lys Gly Val Gln	3408

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CTC ACG GCA CTG AAC TTC AGC GCC GAG CCG GTC AGC GAG ACC ATC TGC Leu Thr Ala Leu Asn Phe Ser Ala Glu Pro Val Ser Glu Thr Ile Cys	3456
CTG CCC GGC GTG GCG CCC GGC CCG GTG GTG GAC ATC ATT CAC GAG AGT Leu Pro Gly Val Ala Pro Gly Pro Val Val Asp Ile Ile His Glu Ser	3504
GTG GAG GGC GAC CTC ACC GAC AAC TGC GAG CTG CAG ATC AAC CTC GAC Val Glu Gly Asp Leu Thr Asp Asn Cys Glu Leu Gln Ile Asn Leu Asp	3552
CCG TAC GAG GGG CTT GCC CTG CGT GTG GTG AGC GCC GCG CCG CCG GTG Pro Tyr Glu Gly Leu Ala Leu Arg Val Val Ser Ala Ala Pro Pro Val	3600
ATC TGA GCGC Ile	3610
CCTCTTCGCG CGCCCCGGGT CCGCCGCTAT AGTGCGCAGC GCCTGGGGCG CGCATTGCCC	3670
TCGCCGTCGA GACCAGCCCG TGTCGTTTAC TTCGCTTTTC CGCCTTGCGC TGCTGCCGCT	3730
GGCGCTGCTT GCCGCACCCG TCTGGGCGCA GACCGCCTGC CCGCCCGGCC AGCAGCCGAT	3790
CTGCCTGAGC GGCAGCTGCC TCTGCGTGCC GGCCGCCGCC AGCGATCCAC AGGCGGTCTA	3850
CGACCGCGTG CAGCGTATGG CTACGCTGGC CCTGCAGAAC TGGATCCAGC AGTCGCGCGA	3910
CCGCCTGATG GCCGGCGGCG TCGAGCCGAT ACCGCTGCAC ATCCGCTCGC AGCTCGAGCC	3970
GTATTTTCGAT CTTGCCGTGC TGGAGAGTGC GCGGTACCGC GTCGGCGACG AGGTGGTGCT	4030
GACTGCCGGC AACACCCTGC TGCGCAACCC GGACGTCAAT GCCGTGACCC TGATCGACGT	4090
CATCGTCTTC CGCCACGAGG AGGATGCCCC GGACAACGTC GCGCTCTGGG CCCATGAGCT	4150
CAAGCACGTC GAGCAATATC TGGACTGGGG CGTCGCCGAG TTCGCCCGGC GCTATACGCA	4210
GGATTTCCGT GCCGTGGAGC GCGCGCCTA TGCGCTGGAG CGTGAGGTGG AAGAGGCCCT	4270
GCGCGAGACG CAGACGCGGC GCTGAGCGAG CTGATCGGTG CTGCTGCCCG CACTGGGCTG	4330
AAGCCCACCA ATGACGCCGG CGAAAACGAA AAACCCCGCC GAGGCGGGGT TTCTGACGCG	4390
GGTTGTGCGG TCAGCTCAGA ACGCCGGGAC CACGGCGCCC TTGTACTTTT CCTCGATGAA	4450
CTGGCGTACT TGCTCGCTGT GCAGCGCGGC AGCCAGTTTC TGCATGGCAT CGCTGTCCTT	4510
GTTGTCCGGA CGGGCGACCA GAATGTTTAC GTATGGCGAG TCGCTGCCCT CGATCACCAG	4570
GGCGTCCTGG GTCGGGTTCA GCTTGGCTTC CAGCGCGTAG TTGGTGTGTA TCAGCGCCAG	4630
GTCGACCTGG GTCAGCACGC GCGGCAGAGT CGCGGCTTCC AGTTCGCGGA TCTTGATCTT	4690
CTTCGGGTTT TCGGCGATGT CTTCCGCGTG GCGGTGATGC CGGCGCCGTC CTTCAGACCG	4750
ATC	4753

## WHAT IS CLAIMED IS:

1. A trehalose synthase protein with the following amino acid sequence:

	Met	Ser	Ile	Pro	Asp	Asn	Thr	Tyr	Ile	Glu	Trp	Leu	Val	Ser	Gln
5					5					10					15
	Ser	Met	Leu	His	Ala	Ala	Arg	Glu	Arg	Ser	Arg	His	Tyr	Ala	Gly
					20					25					30
	Gln	Ala	Arg	Leu	Trp	Gln	Arg	Pro	Try	Ala	Gln	Ala	Arg	Pro	Arg
					35					40					45
10	Asp	Ala	Ser	Ala	Ile	Ala	Ser	Val	Trp	Phe	Thr	Ala	Tyr	Pro	Ala
					50					55					60
	Ala	Ile	Ile	Thr	Pro	Glu	Gly	Gly	Thr	Val	Leu	Glu	Ala	Leu	Gly
					65					70					75
	Asp	Asp	Arg	Leu	Trp	Ser	Ala	Leu	Ser	Glu	Leu	Gly	Val	Gln	Gly
15					80					85					90
	Ile	His	Asn	Gly	Pro	Met	Lys	Arg	Ser	Gly	Gly	Leu	Arg	Gly	Arg
					95					100					105
	Glu	Phe	Thr	Pro	Thr	Ile	Asp	Gly	Asn	Phe	Asp	Arg	Ile	Ser	Phe
					110					115					120
20	Asp	Ile	Asp	Pro	Ser	Leu	Gly	Thr	Glu	Glu	Gln	Met	Leu	Gln	Leu
					125					130					135
	Ser	Arg	Val	Ala	Ala	Ala	His	Asn	Ala	Ile	Val	Ile	Asp	Asp	Ile
					140					145					150
	Val	Pro	Ala	His	Thr	Gly	Lys	Gly	Ala	Asp	Phe	Arg	Leu	Ala	Glu
25					155					160					165
	Met	Ala	Tyr	Gly	Asp	Tyr	Pro	Gly	Leu	Tyr	His	Met	Val	Glu	Ile
					170					175					180
	Arg	Glu	Glu	Asp	Trp	Glu	Leu	Leu	Pro	Glu	Val	Pro	Ala	Gly	Arg
					185					190					195
30	Asp	Ser	Val	Asn	Leu	Leu	Pro	Pro	Val	Val	Asp	Arg	Leu	Lys	Glu
					200					205					210
	Lys	His	Tyr	Ile	Val	Gly	Gln	Leu	Gln	Arg	Val	Ile	Phe	Phe	Glu
					215					220					225

Pro Gly Ile Lys Asp Thr Asp Trp Ser Val Thr Gly Glu Val Thr  
230 235 240

Gly Val Asp Gly Lys Val Arg Arg Trp Val Tyr Leu His Tyr Phe  
245 250 255

5 Lys Glu Gly Gln Pro Ser Leu Asn Trp Leu Asp Pro Thr Phe Ala  
260 265 270

Ala Gln Gln Leu Ile Ile Gly Asp Ala Leu His Ala Ile Asp Val  
275 280 285

Thr Gly Ala Arg Val Leu Arg Leu Asp Ala Asn Gly Phe Leu Gly  
10 290 295 300

Val Glu Arg Arg Ala Glu Gly Thr Ala Trp Ser Glu Gly His Pro  
305 310 315

Leu Ser Val Thr Gly Asn Gln Leu Leu Ala Gly Ala Ile Arg Lys  
320 325 330

15 Ala Gly Gly Phe Ser Phe Gln Glu Leu Asn Leu Thr Ile Asp Asp  
335 340 345

Ile Ala Ala Met Ser His Gly Gly Ala Asp Leu Ser Tyr Asp Phe  
350 355 360

Ile Thr Arg Pro Ala Tyr His His Ala Leu Leu Thr Gly Asp Thr  
20 365 370 375

Glu Phe Leu Arg Met Met Leu Arg Glu Val His Ala Phe Gly Ile  
380 385 390

Asp Pro Ala Ser Leu Ile His Ala Leu Gln Asn His Asp Glu Leu  
395 400 405

25 Thr Leu Glu Leu Val His Phe Trp Thr Leu His Ala Tyr Asp His  
410 415 420

Tyr His Tyr Lys Gly Gln Thr Leu Pro Gly Gly His Leu Arg Glu  
425 430 435

His Ile Arg Glu Glu Met Tyr Glu Arg Leu Thr Gly Glu His Ala  
30 440 445 450

Pro Tyr Asn Leu Lys Phe Val Thr Asn Gly Val Ser Cys Thr Thr  
455 460 465

Ala Ser Val Ile Ala Ala Ala Leu Asn Ile Arg Asp Leu Asp Ala  
470 475 480

Ile Gly Pro Ala Glu Val Glu Gln Ile Gln Arg Leu His Ile Leu  
485 490 495

5 Leu Val Met Phe Asn Ala Met Gln Pro Gly Val Phe Ala Leu Ser  
500 505 510

Gly Trp Asp Leu Val Gly Ala Leu Pro Leu Ala Pro Glu Gln Val  
515 520 525

10 Glu His Leu Met Gly Asp Gly Asp Thr Arg Trp Ile Asn Arg Gly  
530 535 540

Gly Tyr Asp Leu Ala Asp Leu Ala Pro Glu Ala Ser Val Ser Ala  
545 550 555

Glu Gly Leu Pro Lys Ala Arg Ser Leu Tyr Gly Ser Leu Ala Glu  
560 565 570

15 Gln Leu Gln Arg Pro Gly Ser Phe Ala Cys Gln Leu Lys Arg Ile  
575 580 585

Leu Ser Val Arg Gln Ala Tyr Asp Ile Ala Ala Ser Lys Gln Ile  
590 595 600

20 Leu Ile Pro Asp Val Gln Ala Pro Gly Leu Leu Val Met Val His  
605 610 615

Glu Leu Pro Ala Gly Lys Gly Val Gln Leu Thr Ala Leu Asn Phe  
620 625 630

Ser Ala Glu Pro Val Ser Glu Thr Ile Cys Leu Pro Gly Val Ala  
635 640 645

25 Pro Gly Pro Val Val Asp Ile Ile His Glu Ser Val Glu Gly Asp  
650 655 660

Leu Thr Asp Asn Cys Glu Leu Gln Ile Asn Leu Asp Pro Tyr Glu  
665 670 675

30 Gly Leu Ala Leu Arg Val Val Ser Ala Ala Pro Pro Val Ile  
680 685

## 2. A trehalose synthase gene with the following nucleotide sequence:

GATCGCTGGC GTACTGCAGG TAGAGCAGGC GCATCGGCCC CCAGGGCGCA TCGGCCGGCT 60  
 CCGCTGTGCC CTGCTGGTTC ATGAAGCGGA CGAAGCGGCC ATCGCGGAAC CGTGGACGCC 120  
 ATTCGGGGCT GTCCGGGTCG CGGCTGTGCG TGAGCGTGCG CCACAGGTGCG CTGCGAAACG 180  
 5 GCGGACCGCT CCAAAGCGCG CCGTGGATGG GATCGCCGAG CAGTTCGTGC AGTCCCAGG 240  
 AACGTTGCGA ATGCAGCGCG CCGAGGCTCA GGCCATGCAG ATACAGGCGC GGTGGCGGTT 300  
 CGGCCGGCAG TTCGGTCCAG TAGCCATAGA TCTCGGCGAA TAGCGCGCGG GCCACGTCGC 360  
 GGCCGTAGTC GGCCTCCACC AGCAGCGCCA GCGGGCTGTT CAGATAGGAG TACTGCAACG 420  
 CCACGCTGGC GATATCGCCG TGGTGCAAGT ATTCCACTGC GTTCATCGCC GCCGGGTCGA 480  
 10 TCCAGCCGGT ACCGGTGGGC GTCACCAGCA CCAGCACCAG TCGCTCGAAG GCGCCGTGTC 540  
 GCTGCAGCTC GCGCAAGGCC AGACGCGCCC GCTGGCGCGG GGTCTCTGCC GCGCGCAGAC 600  
 CGACGTAGAC GCGAATCGGC TCGAGCGCCG AGCGGCCGCT CAAGACGCTG ATATCCGCCG 660  
 CCGACGGGCC GGAGCCGATG AACTCGCGGC CCGTGCGGCC CAGCTCCTCC CAGCGCAGCA 720  
 ACGAGGCCCG GCTGCCGCTT TTCAGCGCGC AGGCCGGTGG CGCCGTCTCC GGTTCGATCA 780  
 15 GGGCGTCGTA CTGCGCGAAG GATGCGTCCA GCATGCGCAG TGCCCGCGCC GCCAGCACAT 840  
 CGCTGAGCAG CGACCAGAAC AGCGCCAGCG CCACCAGCAC GCCGATCAGC TTGGCCAGGC 900  
 GCCGTGGCAG CACGCGGTGCG GCGTGCCGCG AGACGAAGCG CGACACCAGC CGATACAGAC 960  
 GCGCCAGCGT CAGCAGGATG AGAAAGGTCG CCAGCGCGGT GAGAATGACT TCGAGCAGGT 1020  
 GCGCACTGCT CACCGGCGGC ATGCCCATCA GCGCGCGTAC CGCGTTCTGC CAGCCGGCGA 1080  
 20 CCTGGCTGAG GAAATACCCG GCCAGCAGCA GGCAGCCGAC CGCGATCAGC AGATTGACCC 1140  
 GCTCGCGCTG CCAGCCTGGG CGCTCCGCGA GTTCCAGATA GCGCCACAGC CAGCGCCAGA 1200  
 ACACGCCGAG GCCATAGCCC ACCGCCAGCG CCGCGCCGGC CAGCACGCCC TGGCTCAGCG 1260  
 TCGAGCGCGG CAGCAGCGAT GCGGTCAGCG CCGCGCAGAA GAACAGCGTG CCCAGCAGCA 1320  
 GGCCGAAACC GGACAGCGAG CGCCAGATAT AGAGGACGGG CAGGTGCAGC ATGAAGATCT 1380  
 25 CCGCGGTGCG GTGACGGCGT CGCGCCTCGG CATATCGAGG CGTGTCCGGT CGTGCGGTTT 1440  
 CCGTGATGGT CCGCAGCAGG CCAATCCGAT GCAACGATGG CCGAGCGGCC GACTCAAACG 1500  
 TCTACATTTC CCTAGTGCTG CCGGAACCGA TCGCCG 1536  
 ATG AGC ATC CCA GAC AAC ACC TAT ATC GAA TGG CTG GTC AGC CAG TCC 1584  
 ATG CTG CAT GCG GCC CGC GAG CGG TCG CGT CAT TAC GCC GGC CAG GCG 1632  
 30 CGT CTC TGG CAG CGG CCT TAT GCC CAG GCC CGC CCG CGC GAT GCC AGC 1680  
 GCC ATC GCC TCG GTG TGG TTC ACC GCC TAT CCG GCG GCC ATC ATC ACG 1728  
 CCG GAA GGC GGC ACG GTA CTC GAG GCC CTC GGC GAC GAC CGC CTC TGG 1776  
 AGT GCG CTC TCC GAA CTC GGC GTG CAG GGC ATC CAC AAC GGG CCG ATG 1824

	AAG CGT TCC GGT GGC CTG CGC GGA CGC GAG TTC ACC CCG ACC ATC GAC	1872
	GGC AAC TTC GAC CGC ATC AGC TTC GAT ATC GAC CCG AGC CTG GGG ACC	1920
	GAG GAG CAG ATG CTG CAG CTC AGC CGG GTG GCC GCG GCG CAC AAC GCC	1968
	ATC GTC ATC GAC GAC ATC GTG CCG GCA CAC ACC GGC AAG GGT GCC GAC	2016
5	TTC CGC CTC GCG GAA ATG GCC TAT GGC GAC TAC CCC GGG CTG TAC CAC	2064
	ATG GTG GAA ATC CGC GAG GAG GAC TGG GAG CTG CTG CCC GAG GTG CCG	2112
	GCC GGG CGT GAT TCG GTC AAC CTG CTG CCG CCG GTG GTC GAC CGG CTC	2160
	AAG GAA AAG CAC TAC ATC GTC GGC CAG CTG CAG CGG GTG ATC TTC TTC	2208
	GAG CCG GGC ATC AAG GAC ACC GAC TGG AGC GTC ACC GGC GAG GTC ACC	2256
10	GGG GTC GAC GGC AAG GTG CGT CGC TGG GTC TAT CTG CAC TAC TTC AAG	2304
	GAG GGC CAG CCG TCG CTG AAC TGG CTC GAC CCG ACC TTC GCC GCG CAG	2352
	CAG CTG ATC ATC GGC GAT GCG CTG CAC GCC ATC GAC GTC ACC GGC GCC	2400
	CGG GTG CTG CGC CTG GAC GCC AAC GGC TTC CTC GGC GTG GAA CGG CGC	2448
	GCC GAG GGC ACG GCC TGG TCG GAG GGC CAC CCG CTG TCC GTC ACC GGC	2496
15	AAC CAG CTG CTC GCC GGG GCG ATC CGC AAG GCC GGC GGC TTC AGC TTC	2544
	CAG GAG CTG AAC CTG ACC ATC GAT GAC ATC GCC GCC ATG TCC CAC GGC	2592
	GGG GCC GAT CTG TCC TAC GAC TTC ATC ACC CGC CCG GCC TAT CAC CAT	2640
	GCG TTG CTC ACC GGC GAT ACC GAA TTC CTG CGC ATG ATG CTG CGC GAA	2688
	GTG CAC GCC TTC GGC ATC GAC CCG GCG TCA CTG ATC CAT GCG CTG CAG	2736
20	AAC CAT GAC GAG TTG ACC CTG GAG CTG GTG CAC TTC TGG ACG CTG CAC	2784
	GCC TAC GAC CAT TAC CAC TAC AAG GGC CAG ACC CTG CCC GGC GGC CAC	2832
	CTG CGC GAA CAT ATC CGC GAG GAA ATG TAC GAG CGG CTG ACC GGC GAA	2880
	CAC GCG CCG TAC AAC CTC AAG TTC GTC ACC AAC GGG GTG TCC TGC ACC	2928
	ACC GCC AGC GTG ATC GCC GCG GCG CTT AAC ATC CGT GAT CTG GAC GCC	2976
25	ATC GGC CCG GCC GAG GTG GAG CAG ATC CAG CGT CTG CAT ATC CTG CTG	3024
	GTG ATG TTC AAT GCC ATG CAG CCC GGC GTG TTC GCC CTC TCC GGC TGG	3072
	GAT CTG GTC GGC GCC CTG CCG CTG GCG CCC GAG CAG GTC GAG CAC CTG	3120
	ATG GGC GAT GGC GAT ACC CGC TGG ATC AAT CGC GGC GGC TAT GAC CTC	3168
	GCC GAT CTG GCG CCG GAG GCG TCG GTC TCC GCC GAA GGC CTG CCC AAG	3216
30	GCC CGC TCG CTG TAC GGC AGC CTG GCC GAG CAG CTG CAG CGG CCA GGC	3264
	TCC TTC GCC TGC CAG CTC AAG CGC ATC CTC AGC GTG CGC CAG GCC TAC	3312
	GAC ATC GCT GCC AGC AAG CAG ATC CTG ATT CCG GAT GTG CAG GCG CCG	3360
	GGA CTC CTG GTG ATG GTC CAC GAG CTG CCT GCC GGC AAG GGC GTG CAG	3408

	CTC ACG GCA CTG AAC TTC AGC GCC GAG CCG GTC AGC GAG ACC ATC TGC	3456
	CTG CCC GGC GTG GCG CCC GGC CCG GTG GTG GAC ATC ATT CAC GAG AGT	3504
	GTG GAG GGC GAC CTC ACC GAC AAC TGC GAG CTG CAG ATC AAC CTC GAC	3552
	CCG TAC GAG GGG CTT GCC CTG CGT GTG GTG AGC GCC GCG CCG CCG GTG	3600
5	ATC TGA GCGC	3610
	CCTCTTCGCG CGCCCCGGGT CCGCCGCTAT AGTGCGCAGC GCCTGGGGCG CGCATTGCCC	3670
	TCGCCGTCGA GACCAGCCCG TGTCGTTTAC TTCGCTTTTC CGCCTTGCGC TGCTGCCGCT	3730
	GGCGCTGCTT GCCGACCCCG TCTGGGCGCA GACCGCCTGC CCGCCCGGCC AGCAGCCGAT	3790
	CTGCCTGAGC GGCAGCTGCC TCTGCGTGCC GGCGCGCCGCC AGCGATCCAC AGGCGGTCTA	3850
10	CGACCGCGTG CAGCGTATGG CTACGCTGGC CCTGCAGAAC TGGATCCAGC AGTCGCGCGA	3910
	CCGCTGATG GCCGGCGGCG TCGAGCCGAT ACCGCTGCAC ATCCGCTCGC AGCTCGAGCC	3970
	GTATTTTCGAT CTTGCCGTGC TGGAGAGTGC GCGGTACCGC GTCGGCGACG AGGTGGTGCT	4030
	GACTGCCGGC AACACCCTGC TGCACAACCC GGACGTCAAT GCCGTGACCC TGATCGACGT	4090
	CATCGTCTTC CGCCACGAGG AGGATGCCCC GGACAACGTC GCGCTCTGGG CCCATGAGCT	4150
15	CAAGCACGTC GAGCAATATC TGGACTGGGG CGTCGCCGAG TTCGCCCGGC GCTATACGCA	4210
	GGATTTCCGT GCCGTGGAGC GCCCGGCCCTA TCGCTGGAG CGTGAGGTGG AAGAGGCCCT	4270
	GCGCGAGACG CAGACGCGGC GCTGAGCGAG CTGATCGGTG CTGCTGCCCC CACTGGGCTG	4330
	AAGCCACCA ATGACGCGG CGAAAACGAA AAACCCCGCC GAGGCGGGGT TTCTGACGCG	4390
	GGTGTGCGG TCAGCTCAGA ACGCCGGGAC CACGGCGCCC TTGTACTTTT CCTCGATGAA	4450
20	CTGGCGTACT TGCTCGCTGT GCAGCGCGGC AGCCAGTTTC TGCATGGCAT CGCTGTCCTT	4510
	GTTGTCCGGA CGGGCGACCA GAATGTTTAC GTATGGCGAG TCGCTGCCCT CGATCACCAG	4570
	GGCGTCTCTG GTCGGGTTCA GCTTGGCTTC CAGCGCGTAG TTGGTGTGA TCAGCGCCAG	4630
	GTCGACCTGG GTCAGCACGC GCGGCAGAGT CGCGGCTTCC AGTTCGCGGA TCTTGATCTT	4690
	CTTCGGGTTT TCGGCGATGT CTTCGGCGTG GCGGTGATGC CGGCGCCGTC CTTCAGACCG	4750
25	ATC	4753

3. A recombinant plasmid containing the trehalose synthase gene of claim 1.

4. The recombinant plasmid according to claim 1 which is recombinant plasmid pCJ122.

5. A transformed E. coli with the recombinant plasmid of claim 1.

6. The transformant according to claim 5 in which the recombinant plasmid is pCJ122.

7. A process for producing trehalose which comprises reacting the trehalose synthase enzyme of claim 1 with maltose solution to obtain trehalose.

5 8. A process for producing trehalose which comprises crushing the transformed E. coli of claim 5, centrifuging the crushed bacteria, and reacting the resulting supernatant with maltose solution to obtain trehalose.

10 9. A novel microorganism *Pseudomonas stutzeri* CJ38 that produces trehalose from maltose.

15

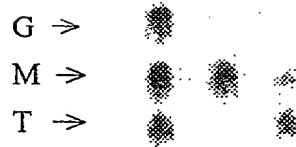
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25

30

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Fig. 1



C CJ38 CJ38  
0h 20h

Fig. 2

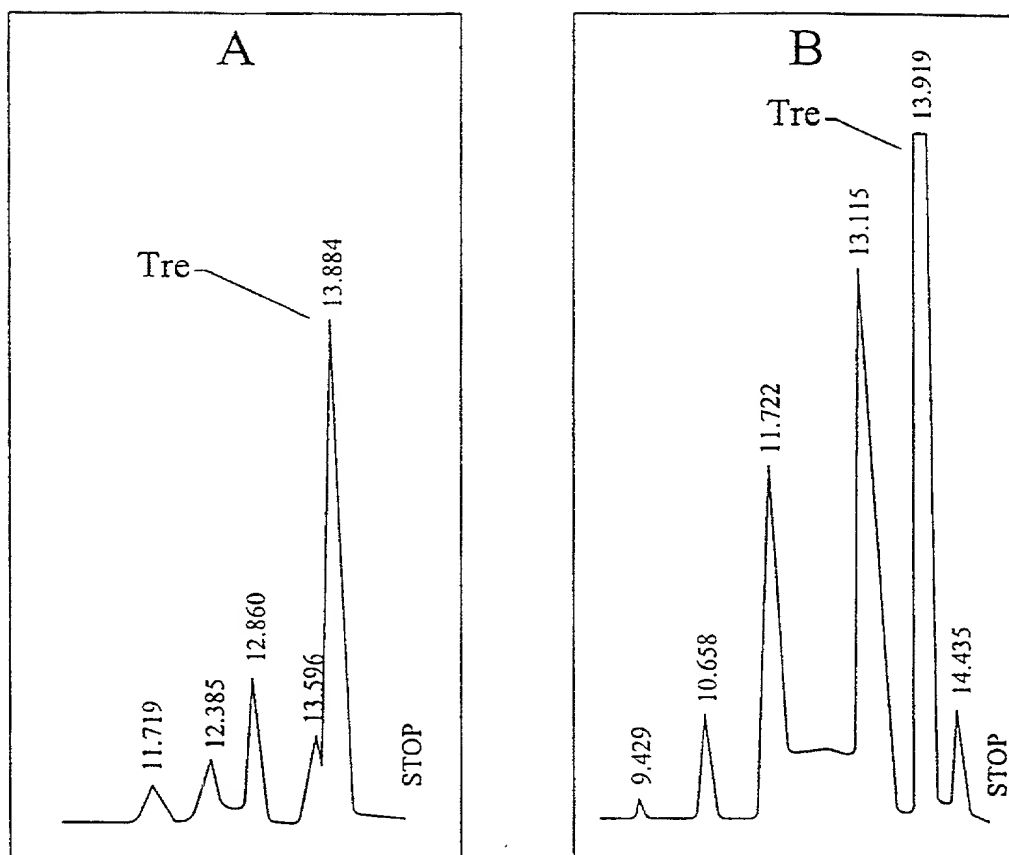
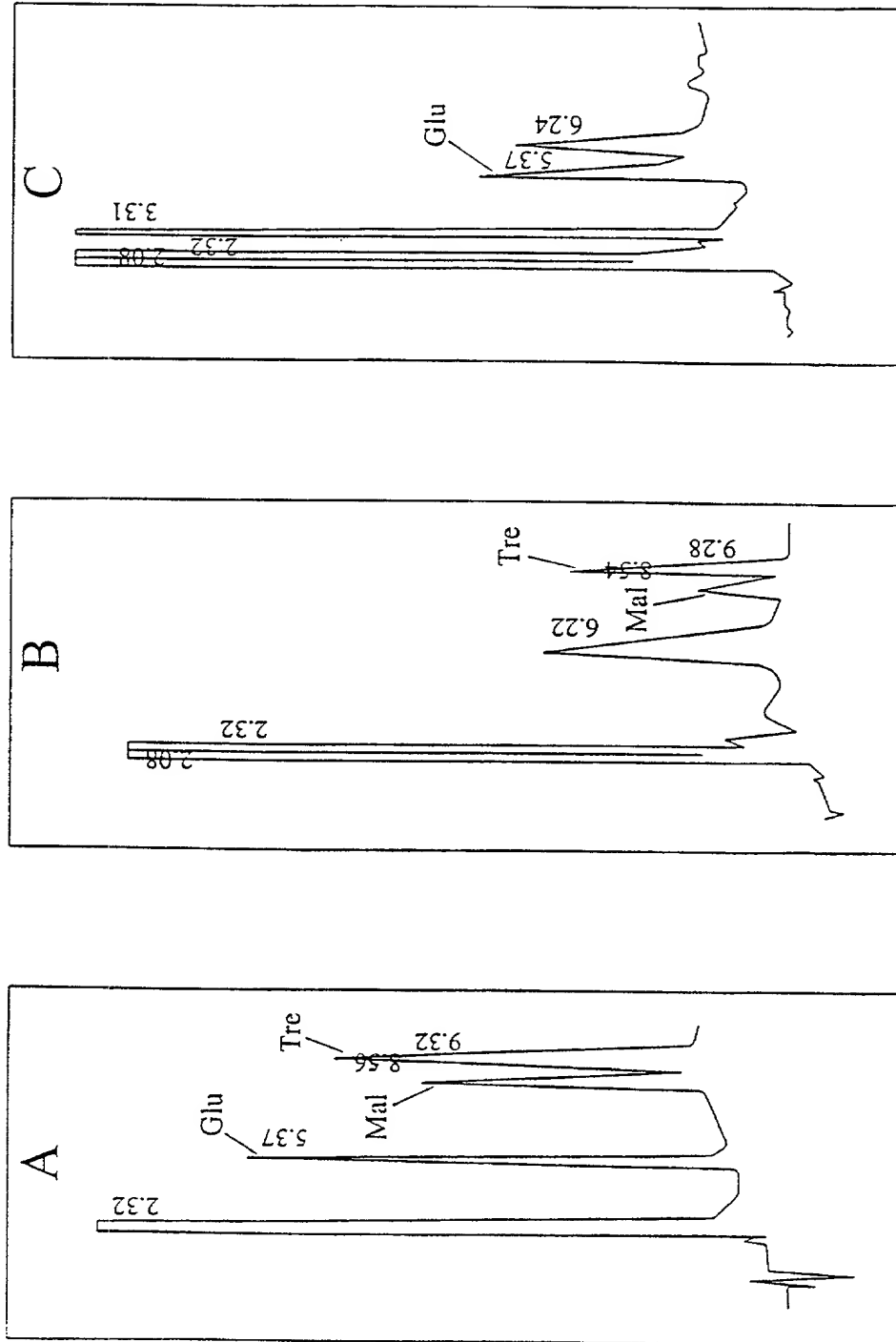


Fig. 3



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Fig. 4

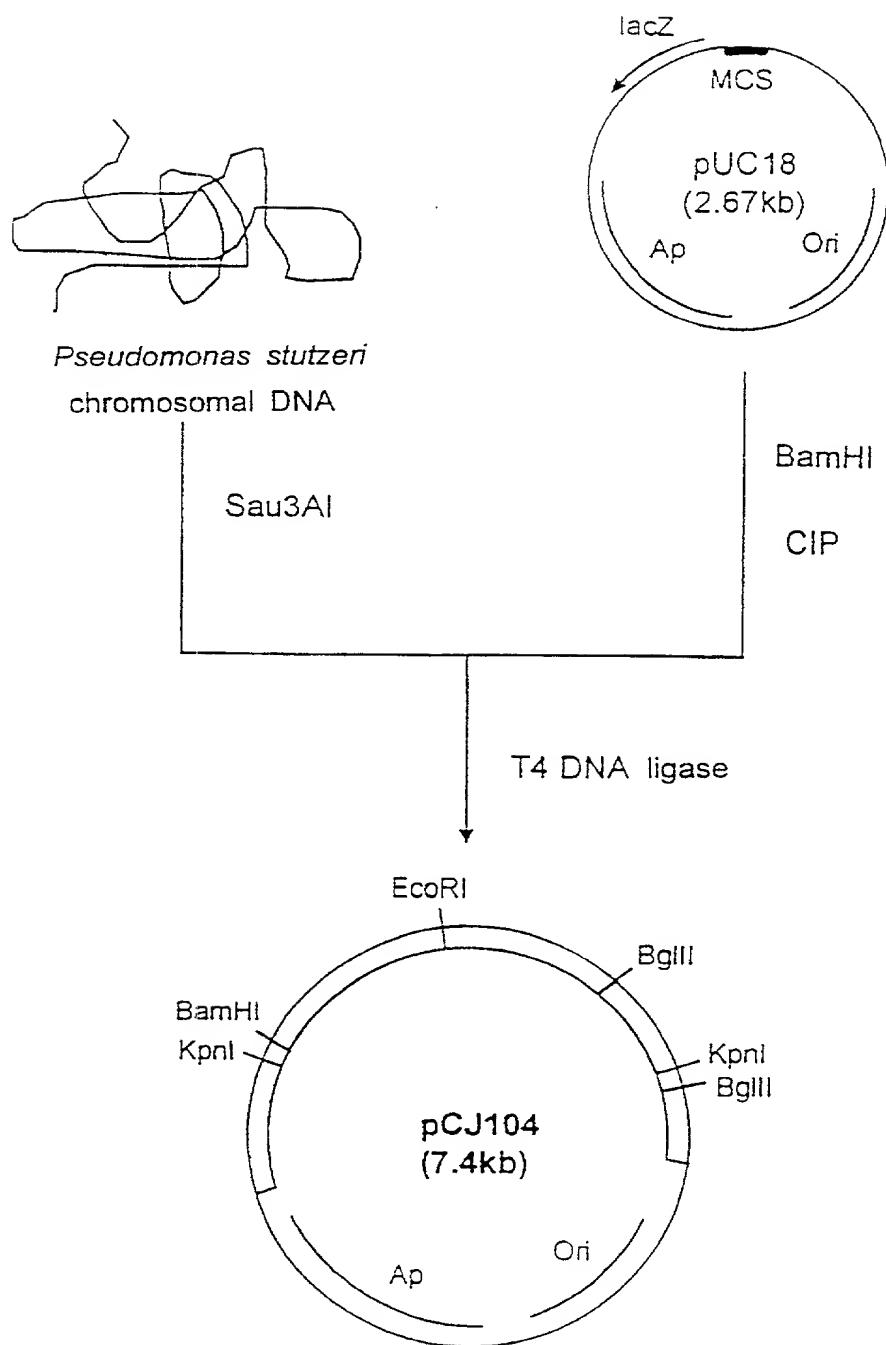


Fig. 5

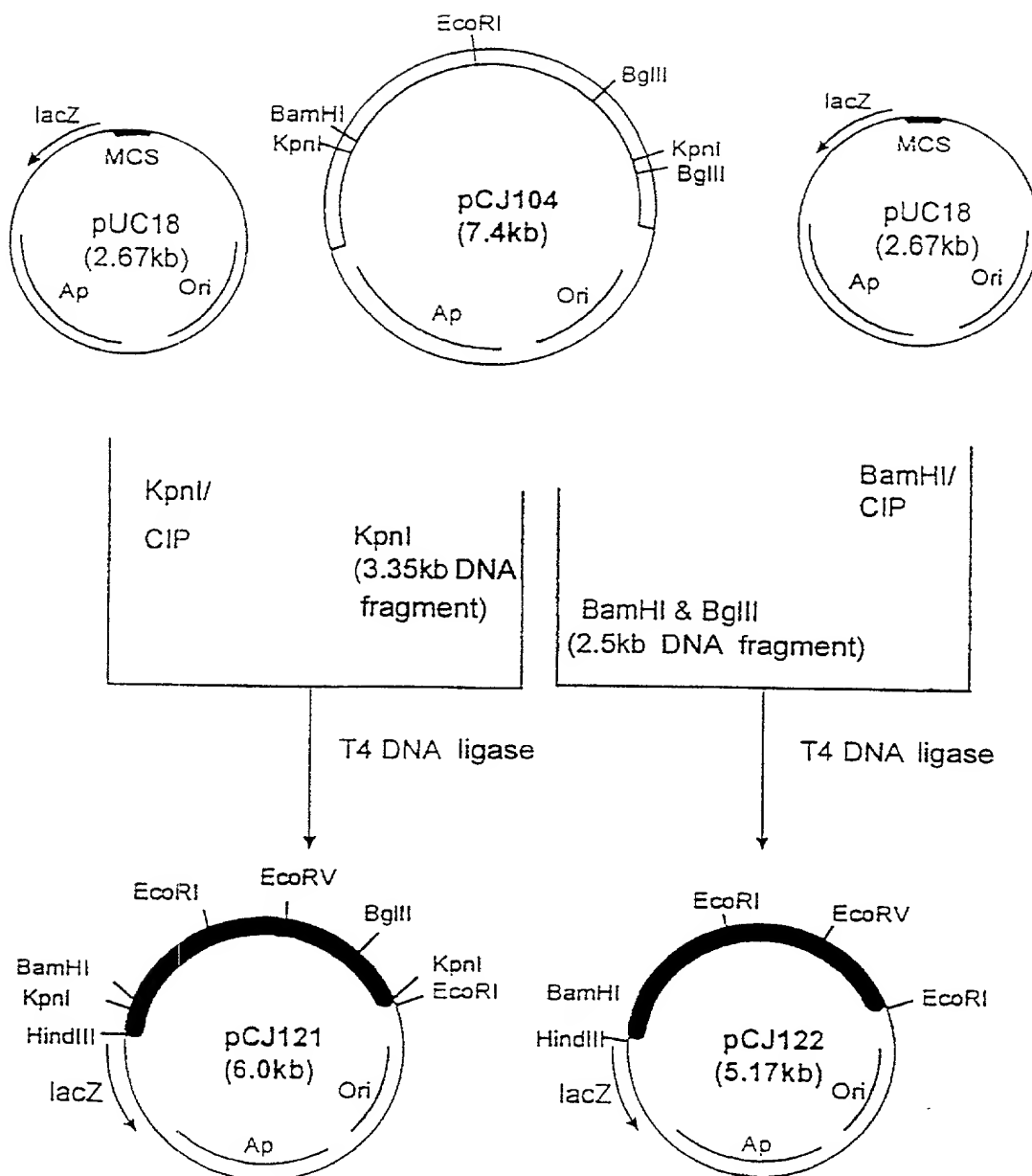
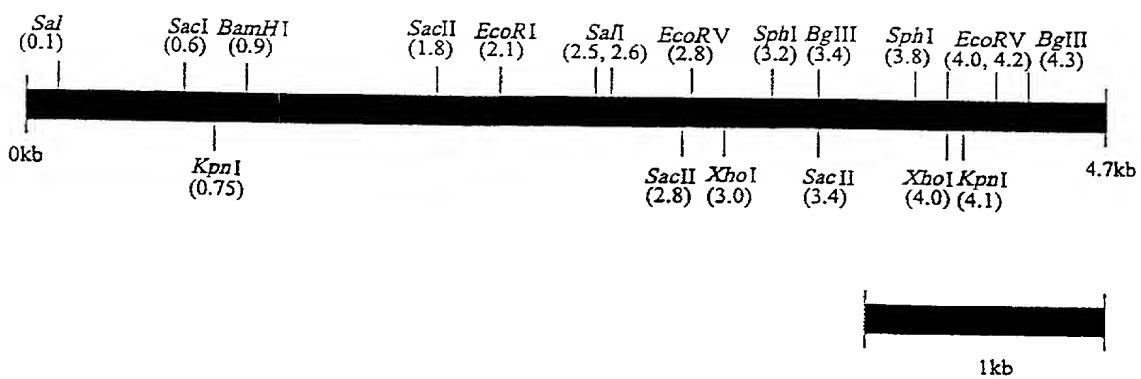


Fig.6



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As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title

"TREHALOSE SYNTHASE PROTEIN, GENE, PLASMIDS, MICROORGANISMS, AND A PROCESS FOR PROTECTING TREHAL

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Attached

the specification of which is attached hereto. If not attached hereto,

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United States Application Number \_\_\_\_\_

and amended on \_\_\_\_\_ (if applicable) and/or

the specification was filed on March 24, 1999 as PCT

International Application Number PCT/KR99/00131 and was

amended under PCT Article 19 on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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Insert Priority  
Information  
(if appropriate)

Prior Foreign Application(s)

Priority Claimed

(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No

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Application(s)  
(if any)

(Application Number)	(Filing Date)
_____	_____
(Application Number)	(Filing Date)
_____	_____

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Country	Application Number	Date of Filing (Month/Day/Year)
_____	_____	_____
_____	_____	_____

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Insert Prior U.S.  
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(if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
_____	_____	_____
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
_____	_____	_____

1599-0206P

Attorney Docket No.

I hereby appoint the practitioners at CUSTOMER NO. 2292 as my attorneys or agents to prosecute this application and/or an international application based on this application and to transact all business in the United States Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the practitioners, unless the inventor(s) or assignee provides said practitioners with a written notice to the contrary.

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(print full name)  
Inventor  
Inventor's Signature  
Inventor's Date This  
Document is Signed

Inventor's Residence  
Inventor's Citizenship

Inventor's Post Office  
Address

Full Name of Second  
Inventor, if any  
see above

Full Name of Third  
Inventor, if any  
see above

Full Name of Fourth  
Inventor, if any  
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Full Name of Fifth  
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Full Name of Sixth  
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KRX  
Republic of Korea

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)

Kunho Apt. #201-103, Hokyedong, Dongan-ku, Anyang, Kyungkee-do 431-080, Korea

Full Name of Ninth  
Inventor, if any  
see above

GIVEN NAME/FAMILY NAME 9-00 Yeong Joong JEON	INVENTOR'S SIGNATURE <i>Yeong Joong Jeon</i>	DATE* Sep. 13, 2001
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Residence (City, State &amp; Country)

Kangdong-ku, Seoul, Korea

CITIZENSHIP

KRX  
Republic of Korea

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)

Jukong Apt. #912-705, Myungil-dong, Kangdong-ku, Seoul 134-070, Korea

Full Name of Tenth  
Inventor, if any  
see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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Residence (City, State &amp; Country)

CITIZENSHIP

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)

Full Name of Eleventh  
Inventor, if any  
see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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Residence (City, State &amp; Country)

CITIZENSHIP

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)

Full Name of Twelfth  
Inventor, if any  
see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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Residence (City, State &amp; Country)

CITIZENSHIP

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)

Full Name of Thirteenth  
Inventor, if any  
see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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Residence (City, State &amp; Country)

CITIZENSHIP

MAILING ADDRESS (Complete Street Address including City, State &amp; Country)